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Mechanosensitive Reflex Input from Distal Colon to Neurons in Pelvic Plexus Ganglia

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of the requirements for

Doctoral degree in Physiology

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MECHANOSENSITIVE REFLEX INPUT FROM DISTAL COLON TO NEURONS IN PELVIC PLEXUS GANGLIA

By

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ABSTRACT

MECHANOSENSITIVE REFLEX INPUT FROM DISTAL COLON TO NEURONS IN PELVIC PLEXUS GANGLIA

By

Jingyang Lin

Neurons in pelvic plexus ganglia (PG) give rise to postganglionic fibers that innervate vascular and visceral smooth muscle of colon, anal-canal, urethra-urinary bladder (UUB) and reproductive organs. Whether these neurons receive mechanoreceptor-mediated sensory input from distal colon and mediate colonic and other visceral reflexes is not known.

The present studies were performed *in vitro* using a guinea-pig colon-PG preparation which consisted of PG, pelvic nerves, hypogastric nerves, colonic-rectal nerves (CRN) and urethra-urinary bladder nerves (UUBN) with or without an attached segment of colon or colon and UUB. Intracellular recordings and intraluminal pressure recordings were used to study electrophysiological properties and synaptic transmission of PG neurons and PG-mediated reflex contractile responses of colon and UUB, respectively.

In isolated PG, neurons were quiescent. Electrical stimulation of the attached nerve trunks elicited nicotinic fast excitatory post-synaptic potentials (f-EPSPs) and/or action potentials (APs) in 70-80% of neurons tested. The inflammatory mediator, human recombinant interleukin-1ß (hrIL-1ß) acting on IL-1 receptors, altered the membrane potential, membrane input resistance and blocked f-EPSPs in some PG neurons. Blockade

of f-EPSPs by hrIL-1ß was due to presynaptic inhibition of acetylcholine release.

When PG were attached to a colon segment via CRN, 27% of neurons exhibited continuous f-EPSPs and APs which were reversibly blocked by hexamethonium and abolished by sectioning the CRN. Passive colon distension increased the frequency of f-EPSPs and APs in active neurons and initiated f-EPSPs and/or APs in quiescent neurons (24%). These results suggest that neurons in PG receive cholinergic mechanosensory input from the colon. Colon application of the inflammatory mediator, bradykinin, sensitized the mechanosensory input. Distension of one colon segment or electrical stimulation of colonic nerves evoked contraction of a second colon segment or of the urinary bladder. Electrical stimulation of UUBN evoked colon contractions, suggesting that neurons in PG mediate excitatory reflexes to the colon and urinary bladder. The reflexes were capsaicin-insensitive, indicating that primary afferents were not involved.

This dissertation showed that neurons in PG receive mechanosensory inputs from distal colon and mediate visceral organ reflexes that are modulated by the inflammatory mediators, bradykinin and hrIL-1ß. The PG-mediated reflexes may be significant in modulating defecation and micturition reflexes physiologically and pathologically.

Zhang, my husband, Dr. Hong Zhang and my father, Dr. Zhongkun Lin for their love and supports. Their attitude toward knowledge and science have made the major influence on my achievement today.

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LIST OF ABBREVIATIONS

Å: Ångström unit, $1x10^{-8}$cm.

Ach: acetylcholine.

4-AP: 4-aminopyridine

APs: action potentials.

ASH: afterspike hyperpolarization

B: urinary bladder.

BK: bradykinin.

BSA: bovine serum albumin

C: coagulating gland.

C_6: hexamethonium.

Ca^{2+}: calcium ion.

CCK: cholecystokinin

CG: celiac ganglia.

CGRP: calcitonin gene related peptide.

Cl^-: chloride ion.

CM: circular muscle.

CN: colonic nerves.

CNS: central nervous system.

CO_2: carbon dioxide.

COL-RECN: colonic-rectal nerves.

cyclic GMP: guanosine 3', 5'-cyclic monophosphate.

DAB: 3, 3'-diaminobenzidine.

DAG: diacylglycerol.

DRG: dorsal root ganglia.

EFS: electrical field stimulation.

ET: endothelin.

f-EPSPs: fast excitatory postsynaptic potentials.

GABA: γ-aminobutyric acid.

H_2O: water.

HGN: hypogastric nerves.

hrIL-1β: human recombinant interleukin-1β.

HRP: horseradish peroxidase.

HTM: high threshold mechanoreceptors.

IBD: inflammatory bowel disease.

IL-1: interleukin-1

IL-1ra: interleukin-1 receptor antagonist.

IMG: inferior mesenteric ganglia.

K^+: potassium ion.

KCl: potassium chloride.

KS: Krebs solution.

L: lumbar root.

LEM: leucocyte endogenous mediator.

LM: longitudinal muscle.

LTM: low threshold mechanoreceptors.

MCF: mononuclear cell factor.

Mg^{2+}: magnesium ion.

MP: myenteric plexus.

mV: millivolts.

nA: nanoAmperes.

Na^+: sodium ion.

NANC: nonadrenergic, noncholinergic neurons.

NE: norepinephrine

NKA: neurokinin A.

NKB: neurokinin B.

NOS: nitric oxide synthase.

NP: nitroprusside.

NPY: neuropeptide Y

O_2: oxygen.

P: prostate gland.

PELN: pelvic nerves.

PG: pelvic plexus ganglia.

PKC: protein kinase C.

rhTNF: recombinant human tumor necrosis factor.

S: sacral nerve.

SCG: superior cervical ganglia.

SMG: superior mesenteric ganglia.

SP: substance P.

SV: seminal vesicles.

TEA: tetraethylammonium.

Tks: tachykinins.

TMB: tetramethyl benzidine dihydrochloride.

TTX: tetrodotoxin.

UUBN: urethra-urinary bladder nerves.

VD: vas deferens.

VIC: vasoactive intestinal contractor.

VIP: vasoactive intestinal polypeptide.

WDM: wide-dynamic range mechanoreceptors.

SUMMARY OF SPECIFIC AIMS

The overall objectives of the present dissertation are to determine the role of pelvic plexus ganglion neurons in the regulation of contractile activity of colon smooth muscle. The experiments proposed will utilize a newly-designed *in vitro* guinea-pig distal colon-pelvic ganglia preparation. The specific aims are:

I. To determine the morphology, the active and passive properties and the component of action potentials of neurons in pelvic plexus ganglia.

II. To study fast excitatory synaptic transmission in pelvic plexus ganglion neurons.

III. To determine whether neurons in pelvic plexus ganglia receive mechanosensory input(s) from the distal colon.

IV. To test the hypothesis that neurons in pelvic plexus ganglia mediate reflex contractions between segments of the colon and between the colon and urinary bladder.

V. To study the interaction of [illegible] mediators with pelvic parasympathetic system.

i. To study the effect of bradykinin on mechanosensory input(s) to neurons in pelvic plexus ganglia.

ii. To determine the effects of human recombinant interleukin-1β on synaptic transmission and on the active and passive properties of neurons in pelvic plexus ganglia.

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GENERAL HISTORY OF THE AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system

If any thing ail a man,
so that he does not perform his functions,
If he have a pain in his bowels
-for that is the seat of sympathy-
he forthwith sets about reforming his world.

Henry D. Thoreau
The Writings of Henry David Thoreau.
Boston: Houghton Mifflin, 1906.

The literary allusion of the bowels as the seat of sympathy, stated by the American novelist Thoreau, underscores that innate sense each of us subconsciously acknowledges of the reactivity of the gastrointestinal tract to rigors of life. Called "sympathy", our reactions to our environment entail a complex nervous system capable of responding acutely and instantaneously to particular circumstances that challenge us. The search for this nervous system and the desire to understand its physiology have constantly occupied that attention of mankind. The "sympathy" nervous system now is called the autonomic nervous system. It is also called the visceral, vegetative, or involuntary nervous system (Langley, 1921).

In the periphery, the autonomic nervous system consists of nerves, ganglia and plexuses that innervate the heart, blood vessels, glands, other visceral organs and smooth muscles. The autonomic nervous system is widely distributed throughout the body and regulates functions, which occur without conscious control.

Afferent fibers from visceral structures are the first link in the reflex arcs of the autonomic nervous system. With certain exceptions, such as peripheral reflexes mediated by the autonomic ganglia and local axon reflexes, most visceral reflexes are mediated through the central nervous system (CNS). The afferent fibers are, for the most part, nonmyelinated and are carried into the cerebrospinal axis by the vagal, pelvic and splanchnic and other autonomic nerves. The cell bodies of visceral afferent fibers lie in the dorsal root ganglia of the spinal nerves and in the sensory ganglia of certain cranial nerves, such as the nodose ganglion of the vagus. Autonomic afferent nerves mediate visceral sensation (including pain and referred pain) in vasomotor, respiratory and viscerosomatic reflexes and in regulation of interrelated visceral activities. Neuroactive peptides including substance P, vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), somatostatin and calcitonin gene related peptide (CGRP) may play a role in afferent neurotransmission.

The efferent nerves of the autonomic nervous system supply all innervated structures of the body except skeletal muscle, which is served by somatic nerves. The efferent part of the autonomic nervous system consists of two large divisions: (1) the sympathetic or thoracolumbar outflow and (2) the parasympathetic or craniosacral outflow. The principal neurotransmitter of all preganglionic autonomic fibers and all postganglionic parasympathetic fibers is acetylcholine (Ach). The adrenergic fibers comprise the majority of the postganglionic sympathetic fibers and the principal neurotransmitter is norepinephrine (NE). However, ATP, a purine nucleotide and neuropeptide Y (NPY), a neuropeptide are also found and can be released as cotransmitters from postganglionic sympathetic terminals.

The sympathetic system is distributed to effectors throughout the body, whereas the distribution of the parasympathetic system is much more limited. Preganglionic sympathetic fibers may traverse a considerable distance of the sympathetic chain and pass through several ganglia before they finally synapse with postganglionic neurons. In

contrast, the parasympathetic system has its terminal ganglia very near to or within the organs innervated and thus can be more circumscribed in its influences.

The integrating action of the autonomic nervous system is of vital importance for the well-being of the organism. In general, the autonomic nervous system regulates the activities of structures that are not under voluntary control and that, as a rule, function below the level of consciousness. Thus, respiration, circulation, digestion, body temperature, metabolism, sweating and the secretion of certain endocrine glands are regulated, in part or entirely, by the autonomic nervous system (Skok, 1973).

Autonomic Ganglia

As early as the second century, the Greek physician Galen (cA. a. d. 129-199) had identified nervous elements extrinsic to the gastrointestinal tract that exerted this particular influence "sympathy" on the abdominal viscera. Galen's account of a visceral nervous system described fine filaments coming off the branches of what is now identified as paravertebral sympathetic chains. Several groups of "swellings" were also observed in the abdominal cavity that are now called autonomic ganglia.. The autonomic ganglia are aggregates of nerve cells with glial cells and connective tissue surrounding them. The extracellular space around the ganglion cells is protected by a blood-ganglia barrier that exists in the major ganglionic capillary system. The ganglia form either separate bodies connected by nerves with each other, with the central nervous system and with peripheral organs (extramural ganglia), or they may form plexuses located within the walls of visceral organs (intramural ganglia). All ganglia receive preganglionic fibers from the central nervous system (CNS) and send postganglionic fibers to effectors such as smooth muscles and glands. The sympathetic ganglia receive preganglionic fibers only from the thoracic and lumbar segments of the spinal cord while the parasympathetic ganglia receive preganglionic fibers from the brain stem and from sacral segments of the spinal cord (Szurszewski and King, 1989).

The cell bodies of sympathetic preganglionic fibers are located in the lateral column of the spinal cord in the *nucleus intermediolateralis*, which spreads from the first thoracic segment to the third lumbar segment. The cell bodies of parasympathetic preganglionic fibers are located in the nuclei of III, IV, IX and X cranial nerves and in sacral segments of the spinal cord (Szurszewski and King, 1989).

Three types of pathways pass through the ganglia. The most common pathway is "centrifugal pathway", present in all autonomic ganglia of vertebrates and invertebrates. In autonomic ganglia of this pathway, preganglionic fibers transmit impulses to many neurons located either in the same ganglia or in several ganglia, and then the impulses spread from postganglionic fibers to the peripheral organs. The second type of pathway is the "peripheral reflex pathway", present in the intramural ganglia of the gastrointestinal tract, in the inferior mesenteric ganglia and in the celiac-superior mesenteric plexus. The impulses arising in the visceral receptors, whose cell bodies are located in the visceral organs, reach the ganglia through afferent fibers and may be transmitted synaptically to ganglion neurons sending their axons to peripheral organs. There is a large number of these neurons which receive both preganglionic and peripheral reflex innervation, and integration between these pathways occurs in the ganglia. The third type of pathway is "centripetal pathway". Traditionally, this pathway consists of mostly afferent fibers of central origin crossing the ganglia without making synaptic contacts within it. However, in the late 1970's, the presence of synaptic contact of this pathway with neurons in the ganglia has been suggested in the inferior mesenteric ganglia (Skok, 1973).

Two lines can be definitely traced in the development of autonomic ganglia from lower forms of vertebrates to higher forms. While unipolar neurons are the only type in amphibian sympathetic ganglia, multipolar neurons are the typical sympathetic ganglion neurons in birds and mammals. The number of nerve cells in the ganglia increases from lower forms of vertebrates to higher forms of vertebrates and the increase in number of

neurons is greater than the increase in number of preganglionic fibers. These processes are presumably related to more complicated organization of the ganglia in higher vertebrates (Skok, 1973).

Innervation of the Gastrointestinal Tract

Gastrointestinal functions are controlled by a hierarchy of humoral, neuronal and myogenic mechanisms. The neural control of the gastrointestinal tract is provided by the parasympathetic and the sympathetic divisions of autonomic nervous system and enteric nervous system. The subdivision is based on the location of the ganglia and connections with the central nervous system. The parasympathetic and sympathetic nerves supply the extrinsic innervation of the gut. The enteric nervous system comprises the intrinsic innervation of the gut.

Parasympathetic innervation The preganglionic neuronal cell bodies of the parasympathetic division are located in the medulla of the brain and in the sacral region of the spinal cord. Peripheral ganglia of the parasympathetic division are positioned within or at the boundaries of the organ system they innervate (Fig. 1a). The preganglionic parasympathetic neurons are cholinergic and their axons terminate on neurons in the intramural plexuses and/or neurons in parasympathetic ganglia. The postganglionic parasympathetic neurons and enteric neurons provide the final common pathway to the muscle for either long reflexes *via* the parasympathetic nerves and enteric neurons or short reflexes within the plexus *via* enteric neurons, respectively (Langley, 1921).

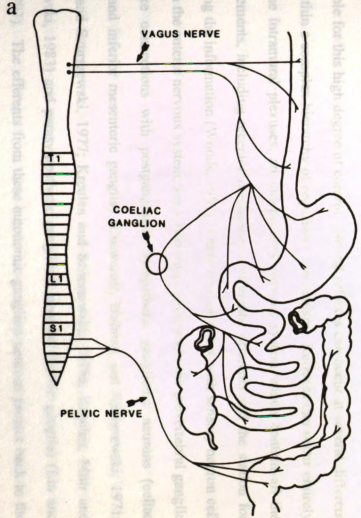
The efferent parasympathetic innervation of the upper gastrointestinal tract is provided by the *nucleus ambiguous* and the dorsal motor nucleus of the vagus in the *medulla oblongata*. The vagus nerve provides parasympathetic innervation to the upper gastrointestinal tract up to the mid-transverse colon. The vagus nerves branch soon after entering the abdomen, with major trunks supplying the stomach and liver. The nerves

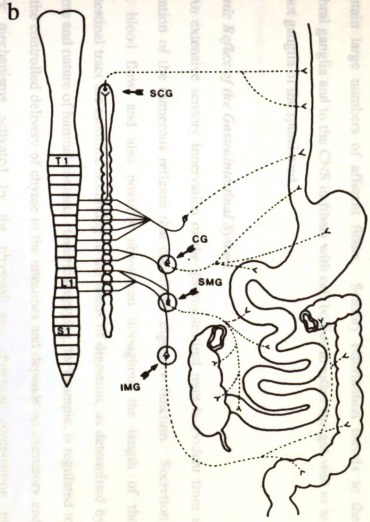
destined for more distal regions travel predominantly in the celiac branch of the vagus nerves and reach the intestines via the paravascular nerve bundles. Only 10% of the abdominal vagal fibers are efferent. The remaining 90% are afferent fibers whose cells bodies lie in the nodose and jugular ganglia and transmit sensory information to the brain (Grundy, 1989). In the lower areas of the gut, the parasympathetic outflow originates from the anterior columns of the sacral spinal cord segments 2, 3 and 4. In most species, these nerves join with sympathetic and afferent fibers to form the pelvic plexus in which the two groups of nerve fibers cannot be separated. The postganglionic fibers originating from pelvic plexus ganglia innervate the anorectal area, the descending colon and the left half of the transverse colon.

Sympathetic innervation The cell bodies of preganglionic neurons of the sympathetic division are located in the intermediolateral cell columns of the spinal cord between the 5th thoracic and 3rd (or 4th in man) lumbar segments. Peripheral ganglia of the sympathetic division are organized in chains of paravertebral ganglia positioned along either side of the spinal cord and prevertebral (celiac, superior, and inferior mesenteric) ganglia within the abdomen (Fig. 1b). The preganglionic neurons in these pathways are cholinergic and they form nicotinic synapses on post-ganglionic neurons, which are adrenergic. Preganglionic fibers to the prevertebral ganglia pass through the paravertebral sympathetic chain without synapsing. They form the thoracic and lumbar splanchnic nerves which terminate in the prevertebral ganglia. Bundles of postganglionic sympathetic nerves and some preganglionic parasympathetic fibers from the celiac branch of the vagus nerve form paravascular nerves which accompany the arteries to the effector organ. The levels of regions of the bowel determine the particular ganglia that supply them. Most efferent adrenergic fibers innervating the gastrointestinal tract terminate on the vasculature and on enteric neurons (Norberg, 1964). A few adrenergic fibers extend into the mucosal villi and around the mucosal glands. Except for sphincteric regions in some species, direct innervation of the smooth muscle is sparse or

VAGUS NERVE
DORSAL GANGLION
PELVIC NERVE

Figure 1. a: Parasympathetic innervation of the gastrointestinal tract. The figure shows only the preganglionic efferent supply. The postganglionic parasympathetic neurons (not shown) are located intramurally or close to the innervated organ. b: Sympathetic innervation of the gastrointestinal tract. Preganglionic neurons are represented by solid lines; post-ganglionic sympathetic neurons are shown as broken lines. SCG=superior cervical ganglia; CG=celiac ganglia; SMG=superior mesenteric ganglia; IMG=inferior mesenteric ganglia. (After Jan Tack, 1992)


a
VAGUS NERVE
T1
L1
S1
COELIAC
GANGLION
PELVIC NERVE


b
SCG
T1
L1
S1
CG
SMG
IMG

absent (Jacobowitz, 1965; Gillespie and Maxwell, 1971). Sympathetic nerves bundles also contain large numbers of afferent fibers. Sensory information travels to the prevertebral ganglia and to the CNS in fibers with cell bodies in either the gut wall or in dorsal root ganglia of the spinal cord.

Autonomic Reflex of the Gastrointestinal System

An extensive sensory innervation of the gastrointestinal tract is evident from a consideration of the numerous reflexes that coordinate digestive function. Secretion, motility, blood flow, and also possibly absorption throughout the length of the gastrointestinal tract are modified according to the needs of digestion, as determined by the volume and nature of luminal contents. Gastric emptying, for example, is regulated to provide the controlled delivery of chyme to the intestines and depends on excitatory and inhibitory mechanisms activated by the physical and chemical composition of gastrointestinal contents (Cooke, 1975). Hormones as well as neural reflexes are responsible for this high degree of control. Neural reflexes can occur at several different levels within a complex hierarchy of regulatory mechanism. Reflexes can occur entirely within the intramural plexuses, an independent nervous system which contain all the reflex elements, including receptor mechanisms, afferents, efferents and the station for integrating the information (Woods, 1981). Other type of reflex with sensory neuron cell bodies in the enteric nervous system, send afferent projections to the prevertebral ganglia and make connections with postganglionic sympathetic ganglionic neurons (celiac ganglia and inferior mesenteric ganglia) (Crowcroft, Holman and Szurszewski, 1971; Weems and Szurszewski, 1977; Kreulen and Szurszewski, 1979a; Kreulen, Muir and Szurszewski, 1983) and parasympathetic ganglionic neurons (pelvic ganglia) (Lin and Krier, 1993). The efferents from these autonomic ganglion neurons project back to the wall of the gastrointestinal tract. The establishment of this reflex pathway has consequently changed our view of the prevertebral ganglia from that of mere relay

stations on the sympathetic and parasympathetic pathways to integrative centers controlling the abdominal viscera. The decentralized gut is therefore capable of complex reactions mediated through peripheral reflexes (Kreulen and Szurszewski, 1979; King and Szurszewski, 1984; Lin and Krier, 1994), which accounts for the often minor and transient consequences of extrinsic denervation, because the peripheral controls are able to adapt to the loss of the extrinsic supply (Grundy, 1981). Finally, the third type of reflexes with sensory neuron cell bodies in external ganglia and connect the abdominal viscera with the CNS. Neural activity in these sensory fibers rarely reaches consciousness; it is more involved in regulatory processes. However, sensations do arise from the gastrointestinal tract. Under normal circumstances, these sensations are generally vague and poorly localized, but can become intense under pathological conditions. The afferent fibers largely follow the same route to the central nervous system as the efferent fibers leaving it; thus they can be divided broadly into parasympathetic and sympathetic afferent fibers. The cell bodies of vagal afferents lie in the superior and inferior (jugular and nodose) vagal ganglia and input to the brain stem. Afferent fibers in the splanchnic and pelvic nerves have their cell bodies in the dorsal root ganglia of the spinal cord and input to the cord through the dorsal roots (Grundy and Scratcherd, 1989).

The Defecation Reflexes

Defecation occurs by coordinated contractions and relaxations of smooth and skeletal muscles of the colon, rectum, anal sphincters and pelvic floor. It is a complex act involving the integration of somatic and autonomic reflex mechanisms and is dependent on the participation of neural populations located within the wall of the colon and rectum (enteric nervous system) and neuronal populations located at various levels of the neuroaxis (including the spinal cord, brain stem, telencephalon and diencephalon).

Ordinarily, defecation is initiated by defecation reflexes. One of these reflexes is an intrinsic reflex mediated by the local enteric nervous system. This reflex is fortified by another type of defecation reflex, a parasympathetic defecation reflex that involves the sacral segments of the spinal cord. However, based upon the studies that will be described in the present dissertation, reflexes mediated by parasympathetic ganglia, pelvic plexus ganglia, may also be part of the parasympathetic defecation reflex. Despite the defecation reflexes, other effects are also necessary before actual defection occurs.

It is generally considered that the primary stimulus of defecation is distension of the rectum. Receptors located in the wall of the rectum are activated, resulting in reflex relaxation of the internal anal sphincter and simultaneous contractions of the external anal sphincter. Rectal distension also generates sensory information that is transmitted via sacral afferent fibers and ascending afferent pathways in the *intermediolateral funiculus* of the spinal cord (Nathan and Smith, 1953) to cortical and diencephalic centers. Telencephalic and/or diencephalic centers provide descending input to somatic efferents to elicit appropriate postural adjustments. If the urge to defecate is suppressed, descending inputs to motoneurons in the sacral spinal cord mediate voluntary contractions of the external anal sphincter, *puborectalis*, and *levator ani*. Tension in the rectal wall decreases the stimulus for defecation. In contrast, during defecation, inputs to somatic efferents effectively increase intrathoracic and abdominal pressure, which assist evacuation. Increases in intrathoracic and intra-abdominal pressure are mediated by a descent of the diaphragm and closure of the glottis. The squatting position also aids in the increase of intra-abdominal pressure.

Descending projections from the brain stem and hypothalamus to preganglionic neurons of the sacral spinal cord facilitate the principal reflexes that underlie defecation. These include activation of parasympathetic efferent fibers in the distal colon and rectum and inhibition of the excitatory input to the external anal sphincter and pelvic floor muscle. Activation of the sacral parasympathetic outflow facilitates excitatory reflexes,

which results in smooth muscle contraction and the propulsion of fecal contents. Inhibition of motor input to the external anal sphincter and pelvic floor results in relaxation of the skeletal muscle.

CHAPTER 1

MORPHOLOGY AND ACTIVE AND PASSIVE PROPERTIES OF NEURONS IN PELVIC PLEXUS GANGLIA.

Introduction

Begining with the pioneering work of Langley and Anderson (1896a, b), it has been shown that preganglionic fibers in pelvic nerves (arising from the sacral spinal cord) and hypogastric nerves (arising from the lumbar spinal cord) form synapses with neurons in pelvic plexus ganglia (Blackman, Crowcroft, Devine, Holman and Yossemurs, 1969; Crowcroft and Szurszewski, 1971; de Groat and Krier, 1978). These neurons are the origin of post ganglionic parasympathetic fibers that innervate vascular and visceral smooth muscle of colon, anal canal, urethra, urinary bladder and reproductive organs (Langley and Anderson, 1896a,b,c). Thus neurons in the pelvic ganglia provide a site for integrating synaptic inputs arising from preganglionic neurons in the sacral and lumbar regions of the spinal cord. Their postganglionic fibers innervate effector structures in the urethra-urinary bladder, colon-rectum and reproductive organs. Parasympathetic nerve activity from the postganglionic fibers originating from neurons in the pelvic plexus ganglia is considered to play an important role in the control of smooth muscle tone of distal colon and urinary bladder.

Anatomy of pelvic plexus ganglia of male guinea-pig. In the male guinea-pig, the pelvic plexuses are located on both sides of the urinary bladder neck and the distal colon. The pelvic plexus is composed of a dense network of blood vessels, nerve branches and a prominent cluster of nerve cells at the junction of the pelvic and hypogastric nerves (Fig. 2). This nerve cell cluster is named the pelvic plexus ganglia (Wozniak and Skowronska, 1966; Crowcroft and Szurszewski, 1971). Small isolated

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HG.N.
V.D.
V.D.
a
b

Figure 2. Pelvic-hypogastric plexus. Ganglion cells are scattered throughout the region where hypogastric and pelvic nerves intersect. HGN, hypogastric nerves; SV, seminal vesicles; VD., vas deferens; P, prostate gland; C, coagulating gland; B, urinary bladder; pelv. N., pelvic nerves; L, lumbar root; and S, sacral nerve. (After Szurszewski and King, 1989). a: dorsal ventral view. b: lateral view.

a
HG.N.
S.V.
V.D.
V.D.
C
P
b
L3
L4
L5
L6
L7
S1
S2
S3
S4
HG.N.
Pelv. N.
B
P

clusters of cells are also found for a short distance along hypogastric nerves and pelvic nerves. The former ones now are named hypogastric ganglia while the later ones are considered to belong to the pelvic plexus ganglia (Crowcroft and Szurszewski, 1971).

Immunohistochemisty of male guinea-pig pelvic plexus ganglia. In male guinea-pigs, there are three types of ganglion cells, distinguished by their immunohistochemical properties. The majority of cells (70%), which do not contain granular vesicles, are considered cholinergic neurons. The other two types containing either large granular vesicles or granular vesicles of variable size are considered to be adrenergic or peptide containing neurons. Ninety five % of the total axon profiles within the ganglia are cholinergic, the remaining 5% are adrenergic. Ninety-nine % of the synapses are cholinergic whereas 1% are adrenergic (Yokota and Burnstock, 1983). These studies indicate that the majority of neurons and the majority of axon profiles in pelvic ganglia are cholinergic.

Electron microscopy studies of male guinea-pig pelvic plexus ganglia. When viewed under the low power electron microscope, each ganglion contains a variable but small number of ganglion cells. The number was estimated to be about 200 in each ganglia. Ganglion cell bodies, about 20-30 μm in diameter, were tightly packed together within groups with larger tissue spaces between the groups. Within these larger spaces, there were fiber tracts containing both myelinated and unmyelinated axons (0.5-3.0 μm in diameter) (Blackman, Crowcroft, Devine, Holman and Yonemura, 1969).

The narrower spaces between individual ganglion cells within a group were occupied by the capsule cells surrounding each cell body and by small bundles of nerve fibers ensheathed by Schwann cells. Nerve fibers were classified as either preganglionic axons or ganglion cell processes. Clusters of fine ganglion cell processes (0.1-0.3 μm) were also seen within the capsule. At synapses, the pre- and postsynaptic membranes were thickened and spaced 100-150 Å apart for distances up to 5000 Å (Blackman, Crowcroft, Devine, Holman and Yonemura, 1969).

Electrophysiological and pharmacological studies of pelvic ganglia. Due to the close location of the pelvic plexus ganglia to the organs, studies of the electrophysiological properties of neurons in guinea-pig pelvic plexus ganglia have not been done since the late 1960s (Blackman, Crowcroft, Devine, Holman and Yonemura, 1969) and early 1970s (Crowcroft and Szurszewski, 1971). But even after these initial studies, the electrophysiological and pharmacological properties of neurons in pelvic plexus ganglia remained unclear for another decade. Recently, Krier and colleagues described the electrophysiological properties of parasympathetic neurons in cat and rabbit pelvic ganglia and obtained qualitative data regarding voltage-dependent calcium and potassium currents (Nishimura, Krier and Akasu, 1993). Their studies revealed the effects of opioid and vasoactive peptides on synaptic transmission and electrophysiological properties of neurons in pelvic plexus ganglia. Enkephalins were found to inhibit nicotinic neurotransmission in cat colonic ganglion cells by specific interactions with delta-type opioid receptors (Kennedy and Krier, 1987). The vasoactive peptides, endothelin (ET) and vasoactive intestinal contractor (VIC) modulated the sacral parasympathetic outflow to the colon by affecting neurons in the parasympathetic ganglia. In summary, ET and VIC inhibited nicotinic synaptic transmission mediated by nicotinic Ach receptors (Nishimura, Krier and Akasu, 1991), depolarized and then hyperpolarized the membrane potential associated with a decrease and an increase in membrane input resistance of neurons (Nishimura, Akasu and Krier, 1993) and reduced or facilitated voltage-dependent calcium currents (Nishimura, Krier and Akasu, 1991). ET and VIC also caused an inward followed by an outward receptor-operated current in parasympathetic neurons. The currents were mimicked by exogenous guanosine 3', 5'-cyclic monophosphate (cGMP). Both ET and VIC also caused contractions of the colon circular muscle (Nishimura, Akasu and Krier, 1992).

The present studies are focused on the role that pelvic plexus ganglion neurons play in regulation of contractile activity of the colon and are part of a complex study of

the function of pelvic plexus ganglia. The studies described in the present chapter were designed to determine the morphology, the active and passive properties and the components of action potentials of neurons in the male guinea-pig pelvic plexus ganglia. These data will be compared to those obtained in other autonomic ganglia in different mammalian species. These studies served basically as a background investigation for the following studies.

Methods

Experiments were performed on tissues from adult male guinea-pigs (200-500 gm), euthanized by exsanguination following carbon dioxide gas-induced anesthetization. Pelvic plexus ganglia (PG) were dissected *in situ* and placed in a single compartment organ bath. In all preparations, PG were pinned to the floor of a Sylgard-lined organ bath. The organ bath was superfused with a modified Krebs solution (2-3 ml/min) containing (mM): Na^+, 137.4; K^+, 5.9; Ca^{2+}, 2.5; Mg^{2+}, 1.2; Cl^-, 134; HCO_3^-, 15.5; $H_2PO_4^-$, 1.2; glucose, 11.5. The solution was preheated to 37-38 °C at the recording site and equilibrated with 95% O_2 - 5% CO_2 gas mixture. Low Ca^{2+} high Mg^{2+} Krebs solutions contained 0.05 mM Ca^{2+} and 30 mM Mg^{2+}.

Electrophysiological procedures. Intracellular potentials were recorded with microelectrodes filled with 3 M-KCl and a tip resistance ranging from 60-110 MΩ. Membrane potential was recorded using an amplifier with an active bridge circuit that allowed current injection into neurons through the recording microelectrode. Signals from the microelectrode were displayed on an oscilloscope with digitized memory and recorded on a pen-writing chart recorder. Data were stored on a videocassette recorder and the output (individual electronic potentials and action potentials) was plotted with an X-Y plotter.

Horseradish peroxidase (HRP) injection technique. The horseradish peroxidase (HRP)-injection technique was used to study the morphological properties such as soma

diameters and number of processes of pelvic plexus ganglion neurons. Individual neurons in pelvic plexus ganglia were filled by intracellular injection of horseradish peroxidase through the recording microelectrode (5% HRP (Sigma) in 0.5 M KCl/Tris buffer solution). Microelectrodes had a tip resistance of 70-140 MΩ. Horseradish peroxidase was injected into neurons by depolarizing currents (1 - 2 nA, 300 ms duration at 0.5-1 Hz for periods of approximately 15-25 min.). Following a 2-hr time period from the time of HRP injection into the last cell studied, ganglia were fixed in a 1% (w/v) glutaraldehyde/1% (w/v) paraformaldehyde phosphate-buffered fixative for 45 min and stored in ice-cold filtered 0.05 M phosphate buffer at pH 7.2-7.4. Fixation and storage were carried out at 3-5°C.

After fixation, all ganglia to be sectioned was first cryoprotected by sequential equilibration in 10%, 20% and 30% sucrose buffer. Ganglia then were sectioned horizontally at 40 μm on a freezing microtome and mounted on gel coated slides. HRP-labeled neurons were visualized by incubating the tissue with tetramethyl benzidine dihydrochloride (TMB) for 12 to 18 hr at 4°C. This was followed by incubating the tissue in 3,3'- diaminobenzidine (DAB) for 5 min at room temperature (22-25°C) (McRorie, Krier and Adams, 1991). The tissue was rinsed in PBS and dehydrated in serial dilutions of ethanol followed by xylene. All tissue was then coverslipped in Permount and allowed to dry for 24 hr. Sections were viewed under light- and dark-field microscopy at magnifications of 100 and 400 X.

Passive properties. The time constant was measured as the time for electrotonic potential to reach 1-1/e of the maximum. Input resistance was calculated as the ratio of the amplitude of the steady-state electrotonic potential (mV) and the magnitude of the hyperpolarizing current pulse (nA) according to Ohm's law.

Active properties. The characteristics of the action potentials, overshoot (mV), maximum rate of rise (V_{max}, V/sec), threshold potential (mV) and action potential duration at 50% of repolarization (ms), afterspike hyperpolarizing potential duration

(ASH) at 90% (ms) were calculated by a computer program after analog to digital conversion. Action potentials were elicited from cells in response to the injection of depolarizing current pulses across the cell membrane (10 ms pulse duration) (0.1-1.5 nA current magnitude).

Sodium and calcium components of action potentials. Action potentials were elicited by direct depolarizing current injection (25-30 ms duration) (0.05-1.0 nA). Tetrodotoxin (TTX, 0.5-1.0 μM), ω-conotoxin GVIA (300-700 nM), dihydropyridine derivative nifedipine hydrochloride (5-15 μM), substitution of normal Krebs solution to low Ca^{2+} (0.05 mM) high Mg^{2+} (30 mM) Krebs solutions and tetraethylammonium bromide (TEA, 30-60 mM) were used. Drugs were dissolved directly into Krebs solution and were superfused onto the ganglia using a three way stopcock.

Drugs used were horseradish peroxidase (HRP, Sigma) ω-conotoxin GVIA (Peninsula Laboratories), tetrodotoxin (TTX, Sigma), tetraethylammonium bromide (TEA, Sigma), nifedipine or nicardipine hydrochloride (Sigma).

Data were expressed as mean ± SEM. Students *t* test was used for paired comparison and $p<0.05$ was considered as significant results.

Results

Morphological properties of neurons in pelvic plexus ganglia.

A total of 60 neurons from nine male guinea-pig pelvic plexus ganglia were filled by intracellular injection of HRP that appeared in one or more serial sections without apparent damage. These neurons were divided into two types according to morphological criteria. One type had a single long process from the soma and was classified as monopolar neurons (33.0%, 20 of 60) (Fig. 3A-F) with a mean soma diameter of 37.3 ± 0.8 μm (ranged from 26.0-53.0 μm) and mean total length of the process of 401.0 ± 61.0 μm (mean ± SEM, ranged from 55.0-990.0 μm). The second type (67.0%, 40 of 60) had multiple short, broad or fine processes which radiated from

the soma and one long process (Fig. 3G-K). The number of processes in this type of neuron varied from two to eight. Thirty-five % (14 of 40) of these neurons had two processes and were classified as bipolar neurons. Sixty-five % (26 of 40) of the neurons had more than two processes and were classified as multipolar neurons. These neurons had soma diameter of 40.2 ± 1.2 μm (mean ± SEM, ranged from 29.0- 58.0 μm). The mean total length of long process was 373.0 ± 32.5 μm (mean ± SEM, ranged 48.0-570.0 μm) and the mean total length of shorter processes was 171.0 ± 48.4 μm (mean ± SEM, ranged from 16.0-564.0 μm). These data are summarized in Table 1 and show that the majority of neurons in pelvic plexus ganglia of male guinea-pigs were multipolar and bipolar neurons.

Passive and active electrophysiological properties and sodium, calcium components of action potentials of neurons in guinea-pig pelvic plexus ganglia.

The passive and active electrophysiological properties of neurons in pelvic plexus ganglia were tested in 89 neurons from isolated pelvic plexus ganglia of 30 guinea-pigs. These neurons were not spontaneously active and had a resting membrane potential of -56.3 ± 0.3 mV (-42 - - 82 mV, n=89); membrane input resistance of 51.4 ± 2.5 MΩ (21.4 - 121.4 MΩ, n=82); membrane time constant of 6.5 ± 0.3 ms (3 - 18 ms, n=76) and amplitude of action potentials of 73.0 ± 0.5 mV (54 -112 mV, n=79).

Phasic and tonic properties of pelvic plexus ganglion neurons. Intracellular injection of suprathreshold depolarizing currents (5 sec., 0.3-0.5 Hz) elicited two types of responses. In one type of neuron, action potentials occurred for the duration of the constant depolarizing pulse while the second type fired a single (4 of 38, 11%) or a burst (34 of 38, 89%) of action potentials at the onset of the depolarizing current. The response of these neurons was referred to as "tonic discharging" and "phasic discharging", respectively (Fig. 4). Tonic discharging neurons represented 57% (51 of 89) of neurons tested, whereas, the remainder represented phasic discharging neurons. The

Figure 3. Camera lucida drawings of horseradish peroxidase (HRP)-injected neurons in pelvic plexus ganglia of male guinea-pigs. A-F, monopolar neurons with one long process. G-K, multipolar neurons with one long process and two or more shorter processes.

Table 1. *Summary of Morphological Properties of Neurons in Pelvic Plexus Ganglia of Male Guinea-Pigs.*

			Soma Diameter (μm)	Length of Processes (μm) Long Processes	Short Processes
Types of Neurons	Number of Processes	Distribution	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.
Monopolar	1	33.3% (20 of 60)	37.3 ± 0.8	401.0 ± 61.0	
Multipolar	2 to 8	66.7% (40 of 60)	40.2 ± 1.2	373.0 ± 32.5	171.0 ± 48.4

S.E.M., Standard Error of Mean.

passive and active electrical properties of phasic and tonic neurons are listed in Table 2. From HRP-injection experiments, 37% (43 of 116) of neurons injected were phasic discharging neurons while the majority (63%, 73 of 116) of neurons were tonic firing neurons. The tonic and phasic neurons showed similar morphology (size of soma, length and number of processes). The data suggest that the majority of neurons in pelvic plexus ganglia were tonic neurons. There was no statistically significant difference between these two types of neurons in either passive electrophysiological properties or morphology. However, the value of after spike hyperpolarization was marginally significantly different between tonic and phasic neurons.

Sodium and calcium components of action potentials. Action potentials that were evoked by direct depolarizing current injection (15-30 ms duration, 0.05 - 0.6 nA) were abolished by superfusion of the ganglia with a TTX-containing Krebs solution (0.5-1.0 μM) (n=6) (Fig. 5). In the presence of TTX, increasing the strength of the depolarizing current elicited action potentials that had a lower rate of rise and amplitude than normal action potentials. Superfusion of ganglia with a low Ca^{2+} (0.05 mM), high-Mg^{2+} (30 mM) Krebs solution containing TTX (1 μM) (Fig. 5) or a Krebs solution containing TTX (1 μM) and ω-conotoxin (700 nM) (data not shown) reversibly abolished both the action potential and the subsequent afterspike hyperpolarization (n=5).

Experiments were conducted to study the calcium component of the action potentials when potassium currents were blocked by external application of TEA (60 mM) (Fig. 6). Under these conditions, the duration of spike (TEA spike) and afterspike hyperpolarization were prolonged. The action potential duration at 50% repolarization before TEA was 2.1 ± 0.2 ms and duration at 50% repolarization of TEA spike was 4.9 ± 0.3 ms (n=4). Addition of TTX (1 μM) at this time blocked only the initial peak of the TEA spikes. The plateau of TEA spikes was unchanged. Superfusion of a low Ca^{2+}, high Mg^{2+} Krebs solution (n=3) or ω-conotoxin (700 nM, n=3) abolished TTX-resistant TEA spikes (Fig. 6), suggesting that the charge carrier of the TTX-resistant TEA spikes

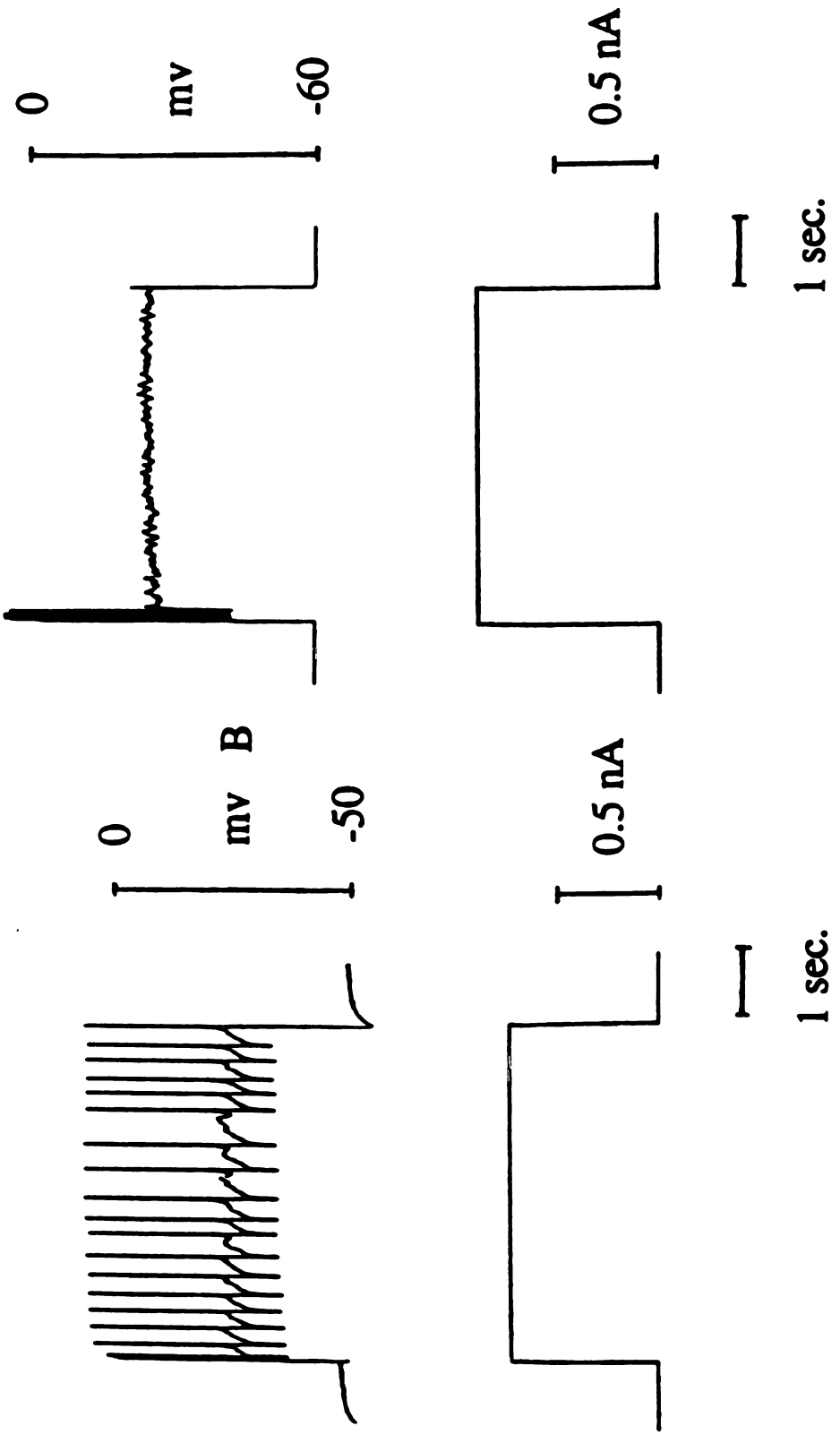


Figure 4. Responses to direct depolarizing current injection (0.7-0.9 nA, 5 sec. duration) of two neurons in guinea-pig pelvic plexus ganglia. A and B: tonic and phasic response respectively.

Table 2. *Electrophysiological properties of phasic and tonic neurons in pelvic plexus ganglia of male guinea-pigs*

	Phasic cells (n=31)	Tonic cells (n=37)
Resting membrane potentials (mV)	-58.3 ± 0.9	-60.3 ± 1.5
Membrane input resistance (MΩ)	49.3 ± 3.2	56.1 ± 3.9
Membrane time constant (t, ms)	6.9 ± 0.6	6.4 ± 0.4
Threshold current to initiate an AP (nA)	0.35 ± 0.11	0.18 ± 0.04
Threshold potential to initiate an AP (mV)	13.3 ± 1.4	11.4 ± 0.9
ASH amplitude (mV)	10.4 ± 1.5	13.0 ± 0.5
ASH 90% duration (ms)	121.8 ± 12.5	140.4 ± 6.5

Values are means ± SEM; AP, action potential evoked by direct depolarizing current injection; ASH, afterspike hyperpolarization

is calcium. In contrast the dihydropyridine derivative, nifedipine (10 μM) did not alter the resting membrane potential or the configuration of the action potentials (n=3) (data not shown).

The data suggest that action potentials in neurons located in pelvic plexus ganglia contain both voltage dependent sodium and calcium currents. The calcium current was carried through ω-conotoxin-sensitive but not nifedipine-sensitive voltage-gated calcium channels.

Figure 5. Sodium and calcium components of the action potential (column A) and spike dependence of the afterspike hyperpolarization (column B) in pelvic plexus ganglion neurons. Aa and Ba, control spike and afterspike hyperpolarization. Application of tetrodotoxin (TTX) (0.5-1 μM) abolished spike discharge (Ab) and afterspike hyperpolarization (Bb). In the presence of TTX (0.5-1 μM), by increasing the intensity of depolarizing current pulse, an action potential with lower amplitude and slower rate of rise was achieved (Ac) while the afterspike hyperpolarization was not different from the control (Bc). Superfusion of low Ca^{2+} (0.05 mM) high Mg^{2+} (30 mM) Krebs solution containing TTX (0.5-1μM) abolished both the TTX-resistant spike (Ad) and afterspike hyperpolarization (Bd). Ae and Be, washed with normal Krebs solution.

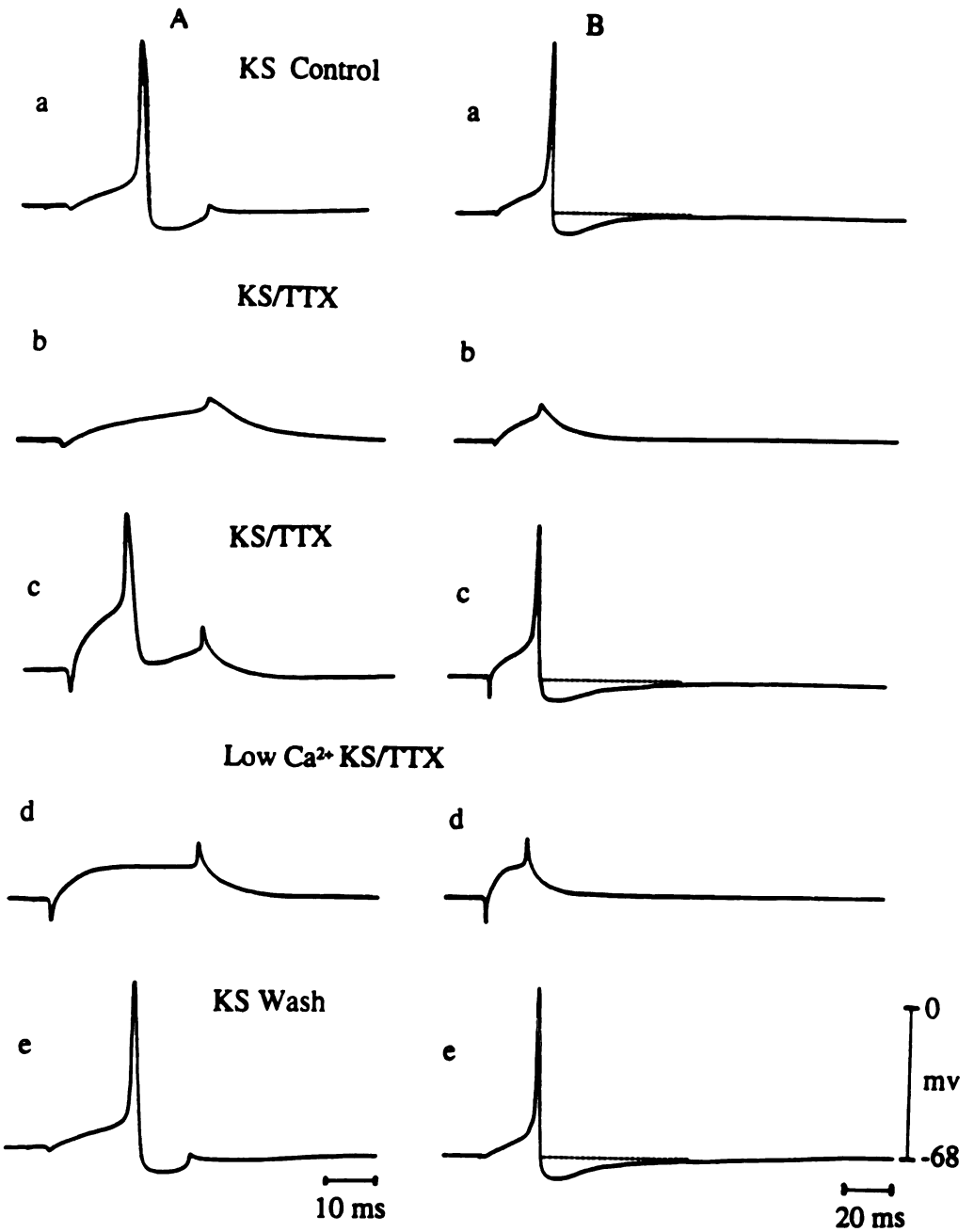
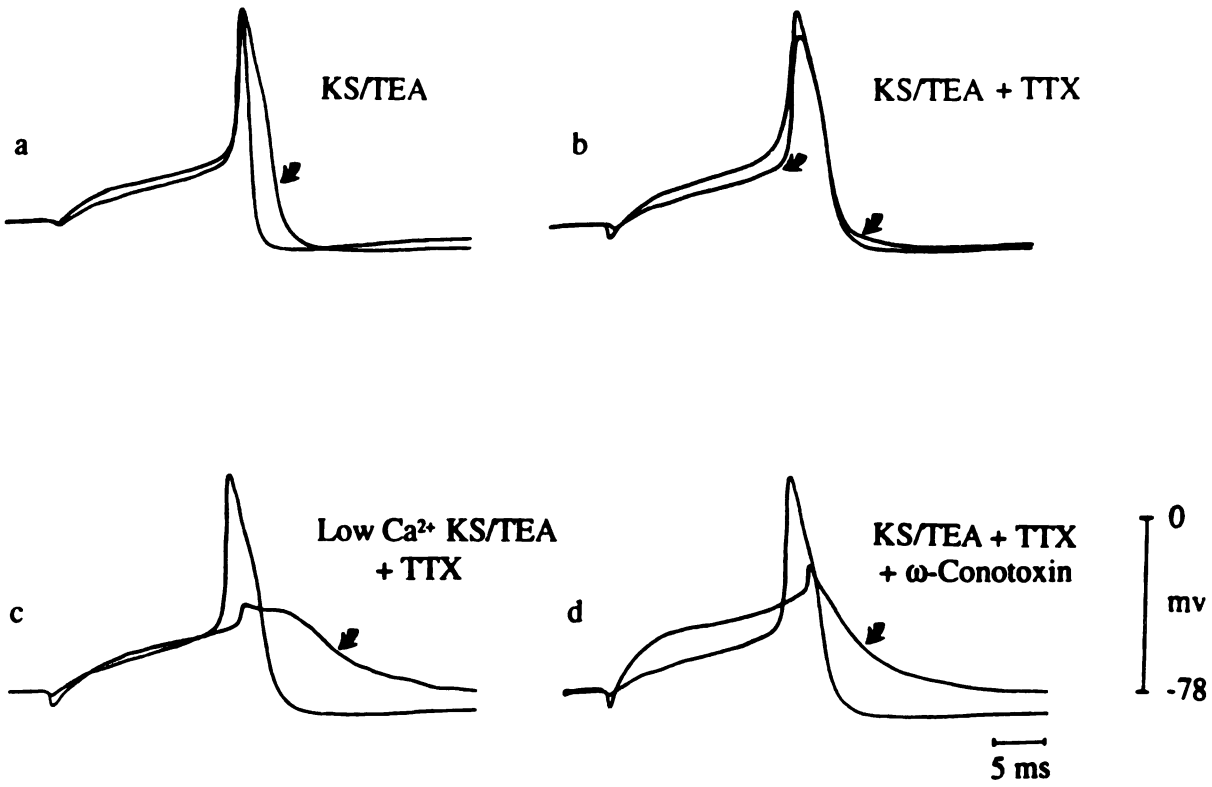

A
B
KS Control
a
a
KS/TTX
b
b
KS/TTX
c
c
Low Ca2+ KS/TTX
d
d
KS Wash
e
e
0
mv
-68
10 ms
20 ms

Figure 6. Effects of tetraethylammonium (TEA, 60 mM) (a), TEA (60 mM) and tetrodotoxin (TTX, 0.5-1μM) (b), low Ca^{2+} (0.05 mM) high Mg^{2+} (30 mM) containing TEA (60 mM) and TTX (0.5-1μM) (c) and TEA (60 mM), TTX (0.5-1 μM) and ω-conotoxin GVIA (700 nM) (d) on action potentials recorded from pelvic plexus ganglion neurons. Action potentials were evoked by brief depolarizing current injection (15-30 ms duration, 0.2-0.5 nA) through recording electrode. Traces of action potentials before and during application of test solutions (arrow) were superimposed.


a
KS/TEA
b
KS/TEA + TTX
c
Low Ca2+ KS/TEA
+ TTX
d
KS/TEA + TTX
+ ω-Conotoxin
0
mv
-78
5 ms

Discussion

Intracellular injection and localization of HRP revealed two morphologically different types of neurons in pelvic plexus ganglia. They are monopolar neurons with only one long process and multipolar neurons with two or more processes. As in other mammalian parasympathetic ganglia such as guinea-pig bronchial parasympathetic ganglia and dog intracardiac ganglia (Myers, Undem and Weinreigh, 1990; Xi, Thomas, Jr., Randall and Wurster, 1991), the majority of neurons in guinea-pig pelvic plexus ganglia are multipolar neurons. In contrast, multipolar neurons in opossum gallbladder parasympathetic ganglia (Bauer, Hanani, Muir and Szurszewski, 1991) are less abundant. The soma diameter resembles the size obtained by Blackman *et al.* (Blackman, Crowcroft, Devine, Holman and Yonemura, 1969) using electron microscopy to measure neurons in guinea-pig pelvic plexus ganglia. The soma diameter and the length of the process of neurons in pelvic plexus ganglia are similar to these parameters defined for neurons in cat lumbar paravertebral ganglia, guinea-pig bronchial parasympathetic ganglia, dog intracardiac ganglia, and opossum gallbladder ganglia (Percy, Walsh and Krier, 1988; Myers, Undem and Weinreigh, 1990; Xi, Thomas, Jr., Randall and Wurster, 1991; Bauer, Hanani, Muir and Szurszewski, 1991). In the opossum gallbladder ganglia, only the long process of monopolar neurons extend beyond the ganglion border in a fiber tract while multipolar neurons had their processes remain inside the ganglia. The authors considered the function of these ganglia to be a simple relay station (Bauer, Hanani, Muir and Szurszewski, 1991). Unlike the neurons in opossum gallbladder ganglia, in guinea-pig pelvic plexus ganglia, both monopolar and multipolar neurons in pelvic plexus ganglia have one or more processes extended beyond the ganglion border and joined in the fiber tracts. We speculate that both types of neurons in guinea-pig pelvic plexus ganglia may be involved in more complicated functions rather than acting as a simple relay station (Bauer, Hanani, Muir and Szurszewski, 1991).

Intracellular recording techniques *in vitro* have previously been used to study the electrophysiological properties of neurons in guinea-pig pelvic plexus ganglia (Blackman, Crowcroft, Devine, Holman and Yonemura, 1969; Crowcroft and Szurszeski, 1971), cat and rabbit pelvic ganglia (Griffith III, Gallagher and Shinnick-Gallagher, 1980; Nishimura, Akasu and Krier, 1991, 1992, 1993) and cat colonic ganglia (Krier and Heartman, 1984). Electrophysiologically, the values for the resting membrane potential, input resistance, time constant, threshold to initiate AP and ASH amplitude and duration resembled those described by Blackman *et al.* and Crowcroft and Szurszeski (Blackman, Crowcroft, Devine, Holman and Yonemura, 1969; Crowcroft and Szurszeski, 1971) for neurons in guinea-pig pelvic plexus ganglia. The passive and active properties are also similar to pelvic ganglion neurons in other species and other mammalian parasympathetic ganglion neurons such as cat pelvic and colonic ganglion neurons (Griffith III, Gallagher and Shinnick-Gallagher, 1991; Krier and Heartman, 1984), guinea-pig bronchial ganglion neurons (Myers, Undem and Weinreigh, 1990), dog intracardiac ganglion neurons (Xi, Thomas, Jr., Randall and Wurster, 1991), guinea-pig gall-bladder ganglion neurons (Mawe, 1990), rat intramural neurons from paratracheal ganglia (Allen and Burnstock, 1990), rat submandibular and cat ciliary ganglia (Skok, 1973). Similar membrane input resistance is characteristic of the similar size of all these neurons. The resting membrane potential in mammalian ganglion neurons ranged from -45 to -80 mV. The wide range of values could be due to differences in the degree of damage to neurons by the microelectrode. The similarities in resting membrane potential usually reflect similar ionic mechanisms underlying the resting membrane potential. The similarities in time constant and capacitance suggest that electrical properties and membrane surface area are similar. The similarities in the passive and active properties of these neurons are not surprising considering the general agreement that neurophysiological values are similar for neurons in parasympathetic ganglia distributed throughout the autonomic nervous

systems of different mammalian species (Gallagher and Shinnick-Gallagher, 1986; Nishi, 1986).

Neurons in pelvic plexus ganglia were characterized as either phasic or tonic based on their response to prolonged superthreshold depolarizing current injection. The present study shows that the majority (60.0%) of neurons in guinea-pig pelvic plexus ganglia exhibit a tonic firing pattern. Phasic and tonic firing patterns have been described previously in several ganglia of guinea-pigs and cats. In cat pelvic ganglia, 93% of neurons were identified as tonic (Griffith III, Gallagher and Shinnick-Gallagher, 1980). The proportion of tonic neurons reported in studies of the celiac and superior mesenteric ganglia varies between about 60% (Kreulen and Szurszewski, 1979) and 35% (Decktor and Weems, 1983). In studies of inferior mesenteric ganglia, either only tonic firing patterns (Crowcroft and Szurszeski, 1971) or about 40% tonic, 60% phasic neurons (Weems and Szurszewski, 1978; Kreulen and Szurszewski, 1979; Julé and Szurszewski, 1983) have been described. Functionally, the prevertebral ganglion neurons have been described to be mainly involved with motility and secretion in the pelvic organs (Langley and Anderson, 1896, a, b, c). whether the proportion of tonic or phasic neurons in certain ganglia will determine the ganglionic function is still unclear. Further studies in inferior mesenteric ganglia have shown that neurons which project in the hypogastric nerves, 87% were tonic and about 12% phasic (Cassell, Clark and McLachlan, 1986). In contrast, 72% of neurons in cat renal ganglia discharged phasically (Decktor and Weems, 1981), as did 96% in the lumbar sympathetic chain (Cassell, Clark and McLachlan, 1986). In the present study, how many of the neurons with different firing patterns may have been projecting in the colonic nerves or urethra-urinary bladder nerves was not determined. However, when the functions served by the components of the outflow from these various ganglia are considered, it appears that these distinctive discharge patterns may provide a basis for the broad classification of sympathetic and parasympathetic ganglion neurons as either vasoconstrictor, vasodilator or visceral.

Tonic and phasic neurons in sympathetic ganglia (Cassell, Clark and McLachlan, 1986; Dektor and Weems, 1983) and enteric ganglia (Nishi and North, 1973) but not parasympathetic ganglia (Griffith III, Gallagher and Shinnick-Gallagher, 1980) have been shown to differ in their active and passive membrane properties. Unlike neurons in sympathetic ganglia which show differences in passive electrical properties that reflect differences in neuronal geometry as well as differences in the population of membrane ion channels opened at resting potential (Cassell, Clark and McLachlan, 1986; Dektor and Weems, 1983), in guinea-pig pelvic plexus ganglia, there is no statistically significant difference between phasic and tonic neurons with respect to passive membrane properties. The active electrical property study showed that after spike hyperpolarization in tonic and phasic neurons was marginally different. However, not all the electrophysiological properties, especially the properties that are considered to be involved in the mechanism of tonic and phasic firing pattern were studied in detail in the present study. Based on the study from inferior mesenteric ganglion neurons (Cassell, Clark and McLachlan, 1986), the mechanism involved in discharge patterns of the two populations of neurons resulted from differences in voltage-dependent potassium channels present in their membranes. The large afterspike hyperpolarization produced by calcium-activated potassium current might contribute to the slow rates of repetitive firing in tonic neurons, while the rhythmic firing of tonic neurons is due to the transient A current (an outward, voltage dependent potassium current). While the high frequency of the initial discharge in phasic neurons can be explained by the absence of an A current, the termination of firing seems to be due to M current (outward, voltage-dependent potassium current which can be blocked by muscarinic receptor agonists). The magnitude of M-current was significantly greater in phasic neurons than in tonic neurons. In some tonic neurons, M-current could not even be detected. Some phasic neurons exhibited two firing patterns with either a single action potential or a short burst of action potentials. These two patterns may resemble the two subclasses of phasic firing neurons

found in guinea-pig inferior mesenteric ganglia (King and Szurszewski, 1988) and cat pelvic ganglia (Griffith III, Gallagher and Shinnick-Gallagher, 1980). Different magnitudes of the M-current may contribute to these two patterns of firing in phasic neurons. Tonic and phasic neurons in guinea-pig pelvic plexus ganglia had similar somal size and dendritic arbors. Monopolar, bipolar and multipolar neurons were all capable of generating both phasic and tonic discharging patterns. This suggests that the capability of generating different patterns of action potentials in neurons of pelvic plexus ganglia is not correlated to the morphological differences.

Action potentials recorded from pelvic plexus ganglion neurons are similar to other mammalian parasympathetic ganglion neurons (Allen and Burnstock, 1987; Mawe, 1990; Xi, Thomas, Jr., Randall and Wurster, 1991; Nishimura and Krier, 1993). The depolarizing phase of the action potential is primarily the result of voltage-activated inward sodium currents, because TTX blocked the action potentials. TTX-resistant spikes were abolished by substituting normal Krebs solution to a low Ca^{2+} high Mg^{2+} Krebs solution or by superfusion with ω-conotoxin GVIA. The appearance of a calcium-dependent spike during superfusion with TTX indicates the presence of a voltage-dependent calcium current in the rising phase of action potentials as well. A high concentration of TEA alone broadened action potentials. Under this condition, the broadened shoulder of the TEA-spike and the TTX-resistant action potential when TTX is added to TEA-Krebs solution may be due to the blockage of a delayed rectifier which allows for opening of voltage-sensitive calcium channels. The calcium current was reversibly blocked by ω-conotoxin and was insensitive to nifedipine, suggesting that for neurons in guinea-pig pelvic plexus ganglia, ω-conotoxin-sensitive N-type but not nifedipine-sensitive L-type voltage-dependent calcium channels are activated during the action potential. The N-type calcium channel has been suggested to be the principal calcium channel subtype through which calcium enters nerve terminals and triggers the release of many chemical transmitter substances. Action potentials in both tonic and

phasic neurons possess a calcium component which is expressed as a prolonged potassium current. This current has been shown to be larger in tonic neurons than in phasic neurons of sympathetic ganglia and has been considered as a major contributor of afterspike hyperpolarization (Cassell, Clark and McLachlan, 1986). However, whether a larger afterspike hyperpolarization is due to a larger N-type calcium current is not known. But if this is true, then more calcium will enter tonic neurons than in phasic neurons through N-type calcium channel. Since calcium entry may also induce long term changes on neuronal properties by activating the signal transduction pathway, this may also be a significant mechanism for different firing pattern in tonic and phasic neurons. In the present study, ASH amplitude and duration are greater in tonic neurons than in phasic neurons, although statistically, these values are marginally significant at $P = 0.05$.

CHAPTER 2
SYNAPTIC TRANSMISSION IN PELVIC PLEXUS GANGLION NEURONS.

Introduction

Preganglionic fibers in sacral ventral roots and pelvic nerves provide synaptic inputs consisting of fast excitatory postsynaptic potentials (f-EPSPs) and/or action potentials, to neurons in cat colonic ganglia (Krier and Hartman, 1984; Nishimura, Krier and Akasu, 1991) and guinea-pig, cat and rabbit pelvic plexus ganglia (Blackman, Crowcroft, Devine, Holman and Yonemura, 1969; Crowcroft and Szurszewski, 1971; McLachlan, 1977; Griffith III, Gallagher and Shinnick-Gallagher, 1980). Pelvic plexus neurons also receive synaptic inputs from preganglionic fibers in lumbar sympathetic nerves (hypogastric nerves). Synaptic potentials evoked by electrical stimulation of the hypogastric nerve and pelvic nerve are blocked by d-tubocurarine, hexamethonium and dihydro-ß-erythroidine (Blackman, Crowcroft, Devine, Holman and Yonemura, 1969; Crowcroft and Szurszewski, 1971; McLachlan, 1977) suggesting that they are mediated by nicotinic acetylcholine receptors.

Although central preganglionic sympathetic (hypogastric) and parasympathetic (pelvic) fibers which provide synaptic inputs to pelvic plexus ganglia have been studied (Blackman, Crowcroft, Devine, Holman and Yonemura, 1969; Crowcroft and Szurszewski, 1971; McLachlan, 1977), it is yet unknown whether neurons in the pelvic plexus ganglia receive synaptic input(s) from peripheral nerve trunks which connect the pelvic ganglia with their effector organs, such as the distal colon-rectum and urethra-urinary bladder. The concept of afferent excitatory synaptic inputs originating from peripheral organs has previously been shown for neurons in sympathetic prevertebral

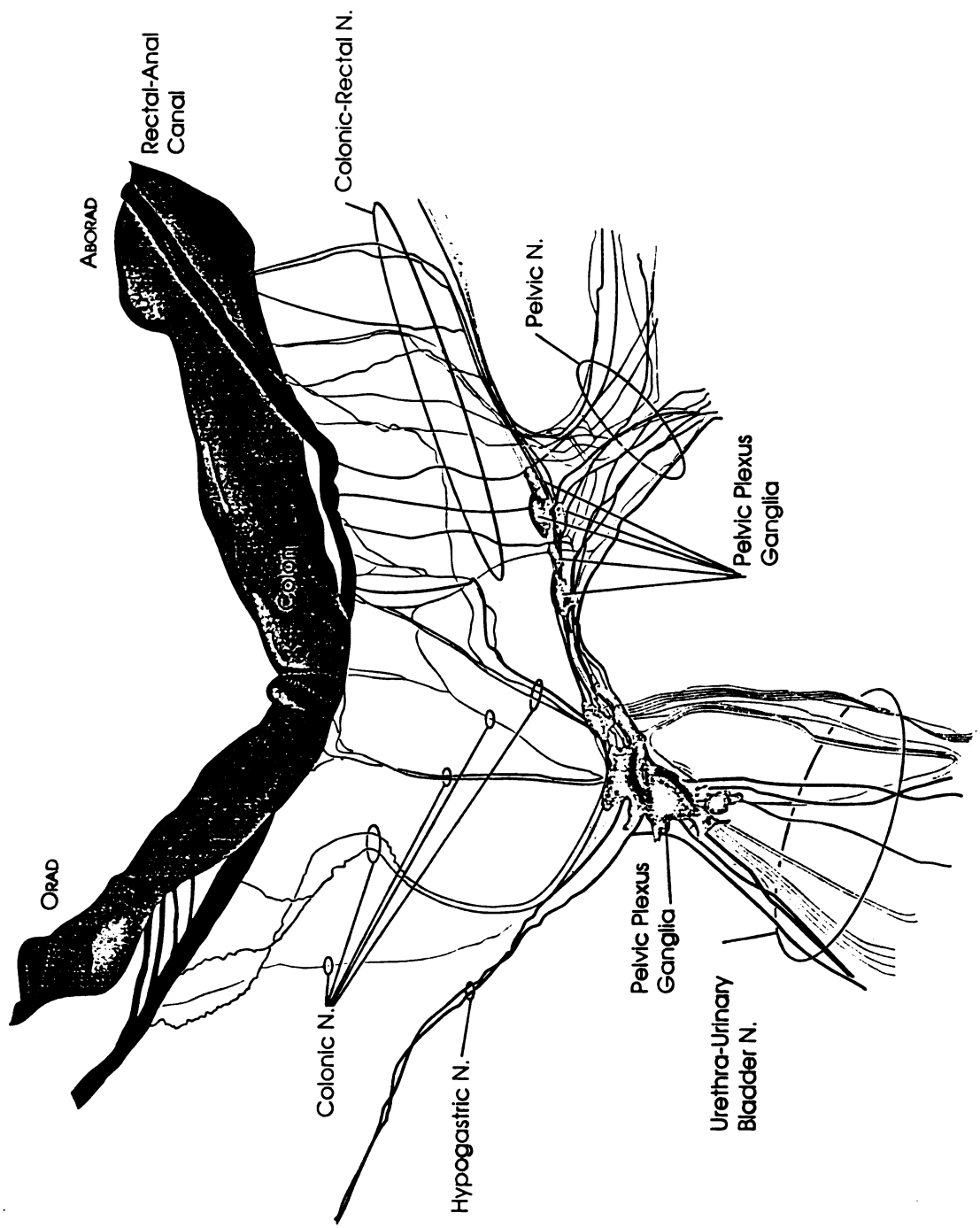
autonomic ganglia (inferior mesenteric, superior mesenteric ganglia and celiac plexus ganglia) of guinea-pigs (Crowcroft, Holman and Szurszewski, 1971; Weems and Szurszewski, 1977; Kreulen and Szurszewski, 1979a; Kreulen, Muir and Szurszewski, 1983).

In the present study, we hypothesize that neurons in pelvic plexus ganglia, like neurons in inferior mesenteric ganglion neurons, receive synaptic inputs originated from peripheral organs. The aim of the present experiments was to determine whether neurons in pelvic plexus ganglia receive peripheral synaptic inputs from nerve fibers that connect distal colon (colonic nerves) and urethra-urinary bladder (urethra-urinary bladder nerves) with pelvic plexus ganglia. The experiments were performed by using an *in vitro* preparation which contained pelvic plexus ganglia and central (pelvic and hypogastric) and peripheral (colonic and urethra-urinary bladder) nerve trunks. The data suggest that in addition to the excitatory synaptic inputs received from central preganglionic nerve fibers, neurons in the pelvic plexus ganglia also receive peripheral synaptic inputs consisting of f-EPSPs and APs in response to electrical stimulation of peripheral nerve trunks. Thirty to forty percent of the responses originated from the peripheral organs while the remainder may be mediated by axon collaterals from efferent and/or afferent fibers in hypogastric and/or pelvic nerves.

Methods

Experiments were performed on adult male guinea-pigs (200-500 gm), euthanized by exsanguination following carbon dioxide gas-induced anesthetization. Pelvic plexus ganglia (PG), together with pelvic nerves (PELN), hypogastric nerve (HGN), urethra-urinary bladder nerves (UUBN) and colonic-rectal nerves (COL-RECN) were dissected *in situ* and placed in a single compartment organ bath. A diagrammatic sketch of the *in vitro* preparation is shown in Figure 7. In all preparations, PG were pinned on Sylgard to

Figure 7. Diagrammatic sketch of lumbar sympathetic and sacral parasympathetic pathways to neurons in pelvic plexus ganglia and to distal colon-rectum of male guinea-pigs. Note: inferior mesenteric ganglia and hypogastric ganglia are not shown.


ORAD
ABORAD
Rectal-Anal Canal
Colonic-Rectal N.
Pelvic N.
Pelvic Plexus Ganglia
Colonic N.
Hypogastric N.
Pelvic Plexus Ganglia
Urethra-Urinary Bladder N.

the floor of the organ bath. The organ bath was superfused with a modified Krebs solution (2-3 ml/min) containing (mM): Na^+, 137.4; K^+, 5.9; Ca^{2+}, 2.5; Mg^{2+}, 1.2; Cl^-, 134; HCO_3^-, 15.5; $H_2PO_4^-$, 1.2; glucose, 11.5. The solution was preheated to 37-38 °C at the recording site and equilibrated with a 95% O_2 - 5% CO_2 gas mixture. Low Ca^{2+} high Mg^{2+} Krebs solutions contained 0.05 mM Ca^{2+} and 30 mM Mg^{2+}.

Electrophysiological procedures. Intracellular potentials were recorded with microelectrodes filled with 3 M-KCl and having tip resistances ranging from 60-110 MΩ . Membrane potentials were recorded using an amplifier with an active bridge circuit allowing current injection into neurons through the recording microelectrode. Signals from the microelectrode were displayed on an oscilloscope with digitized memory and recorded on a pen-writing chart recorder. Data were stored on a videocassette data recorder and the output (individual electrotonic potentials, f-EPSPs, and action potentials) were plotted (superimposed consecutive records) with an X-Y plotter.

Fast-EPSPs and APs were recorded from neurons in response to electrical stimulation of preganglionic fibers. Bipolar platinum stimulating electrodes were positioned on central nerve trunks which connect the pelvic plexus ganglia with the sacral spinal cord (pelvic nerves) and with inferior mesenteric ganglia (hypogastric nerve) and peripheral nerve trunks which connect the ganglia with distal colon-rectum (colonic-rectal nerves) and with urethra-urinary bladder (urethra-urinary bladder nerves). Stimulation was produced by rectangular pulses of 0.3-0.5 ms duration at various frequencies and intensities.

Antidromic potentials were distinguished from synaptic potentials by the occurrence of all-or-none action potentials without a subthreshold synaptic potential and by the occurrence of fast rising potentials during high frequency nerve stimulation that decayed at the rate of the membrane time constant during the injection of hyperpolarizing current pulses (Hartman and Krier, 1984). Also, antidromic potentials are resistant to hexamethonium (Kreulen and Szurszewski, 1979b). Drugs were dissolved directly into

Krebs solution. Solutions were changed by using a three-way stopcock and superfused on ganglia.

Chronic surgical procedures. In five animals, sacral dorsal and ventral roots on one side (S_2 - S_4) and hypogastric nerves on both sides (1 -2 cm below the caudal lobes of the inferior mesenteric ganglia) were transected under ether anesthesia. Following 5 - 27 days of recovery, the animals were reanethestized and surgically prepared for removal of chronically decentralized pelvic ganglia and the associated nerve trunks for the *in vitro* experiments.

Drugs used were hexamethonium bromide (Sigma), dihydro-ß-erythroidine (Sigma), atropine sulfate (Sigma), gamma-aminobutyric acid (GABA, Sigma),

Data were expressed as means ± SEM The significance of differences was assessed by a Chi-Square test for nominal data and $P < 0.05$ was considered significant.

Results

Synaptic potentials and pharmacological studies. Electrical stimulation of preganglionic fibers in hypogastric nerve and pelvic nerves elicited action potentials and/or subthreshold f-EPSPs in neurons of pelvic plexus ganglia. Superfusion of the ganglia with Krebs solution containing hexamethonium (C_6) (10 - 100 μM) (n=180), dihydro-ß-erythroidine (10 - 100 μM) (n=40) and gamma-aminobutyric acid (GABA) (10 - 100 μM) (n=5) or by stimulating nerve trunks at frequencies of 5-15 Hz (n=20) reversibly suppressed orthodromic action potentials and completely abolished fast EPSPs (Fig. 8). Atropine (0.1 - 1.0 μM) (n=14) did not alter orthodromic action potentials or fast EPSPs. Similar responses were also obtained by electrical stimulation of peripheral nerve trunks that connected the pelvic plexus ganglia with distal colon-rectum (colonic-rectal nerves) and with urethra-urinary bladder (urethra-urinary bladder nerves). The data suggest that neurons in pelvic plexus ganglia receive synaptic inputs consisting of f-

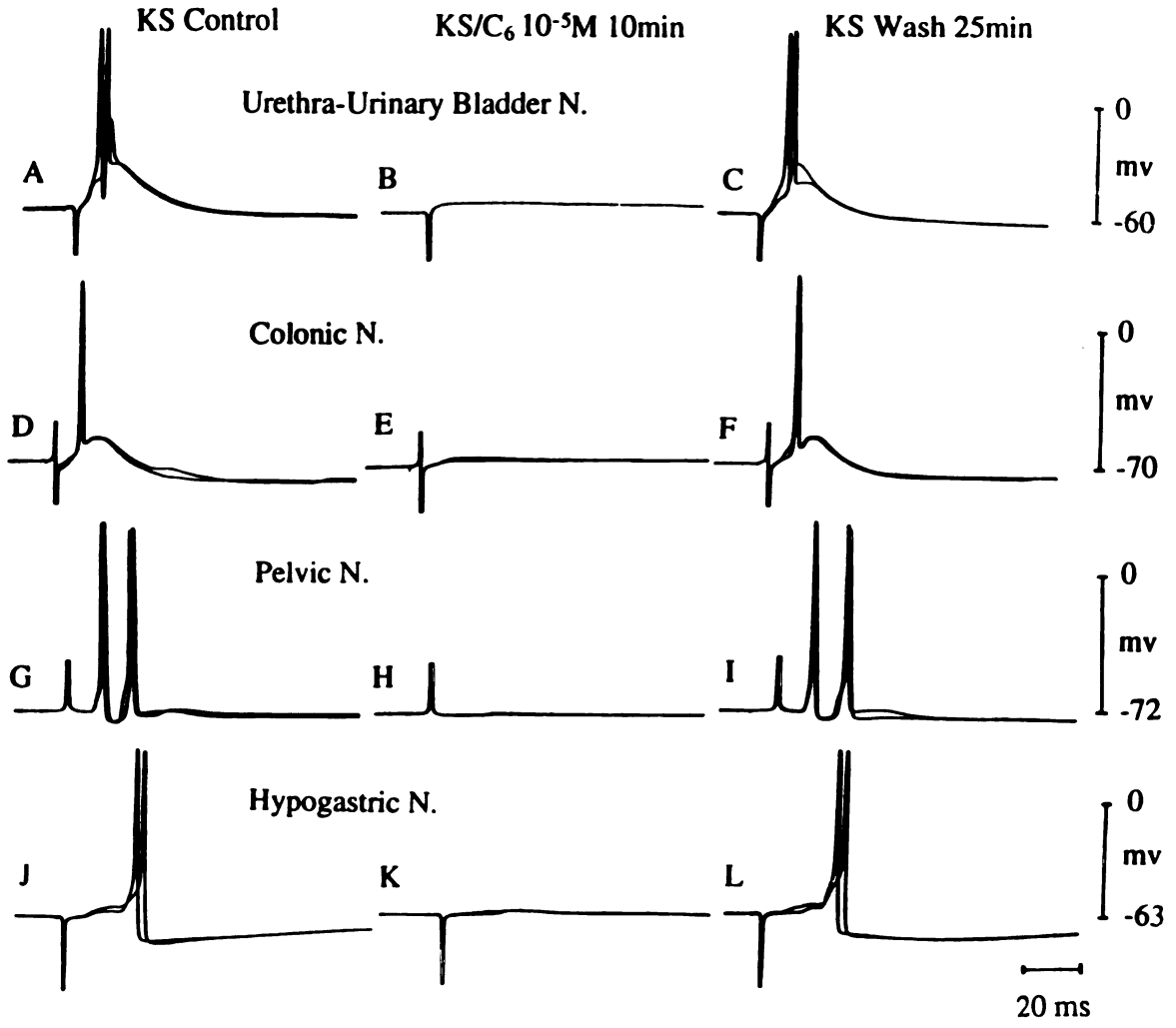


Figure 8. Synaptic responses of neurons in pelvic plexus ganglia during stimulation of urethra-urinary bladder nerves (A-C), colonic-rectal nerves (D-F), pelvic nerves (G-I) and hypogastric nerve (J-L). A, D, G and J, synaptic responses of neurons in Krebs solution (KS). B, E, H and K, synaptic responses of neurons during superfusion of ganglia with a Krebs-hexamethonium (C_6) solution (10 μM). C, F, I and L, synaptic responses of neurons in pelvic plexus ganglia 25 minutes after superfusion of ganglia with Krebs solution (KS). Each panel represents superimposed records of two successive responses to nerve stimulation at maximum intensity.

EPSPs and action potentials not only from central nerve trunks (hypogastric nerve and pelvic nerves) but also from peripheral nerve trunks (colonic-rectal nerves and urethra-urinary bladder nerves). The synaptic inputs were mediated by acetylcholine nicotinic receptors.

Figure 9a shows the distribution of synaptic input(s) to neurons in pelvic plexus ganglia from electrical stimulation of the nerve trunks indicated. Electrical stimulation of hypogastric, pelvic, colonic-rectal and urethra-urinary bladder nerves, elicited synaptic responses in 84 % (205 of 245), 80 % (102 of 128), 67 % (108 of 161) and 80 % (109 of 135) of neurons tested, respectively.

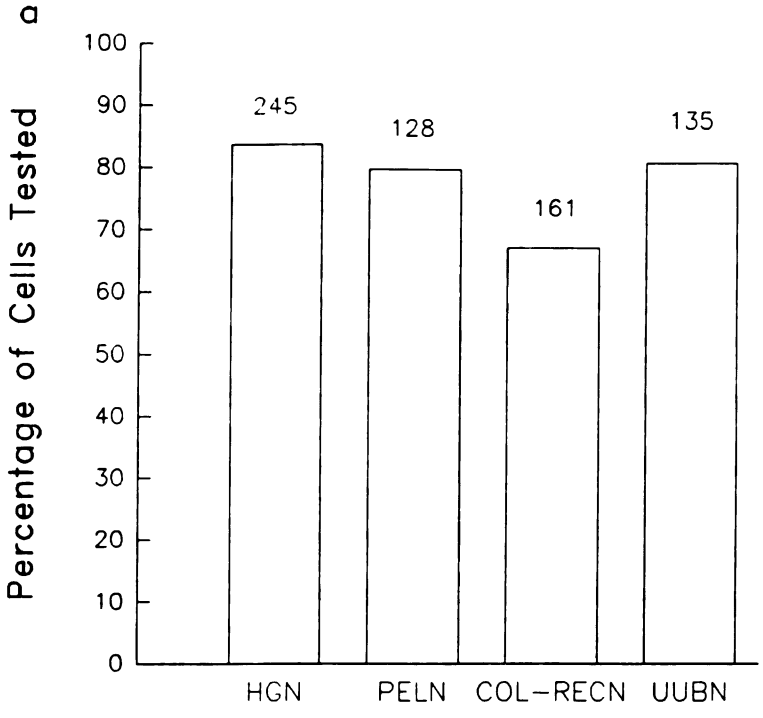
The data indicate that majority of neurons in pelvic plexus ganglia received synaptic inputs not only from central nerve trunks (hypogastric nerve and pelvic nerves) but also from peripheral nerve trunks (colonic-rectal nerves and urethra-urinary bladder nerves).

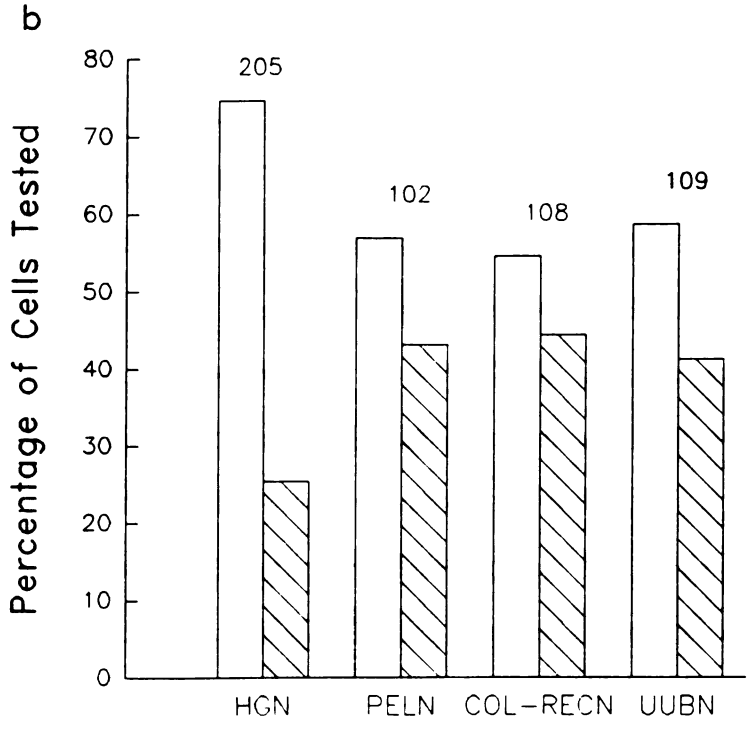
Pattern of synaptic potentials. Electrical stimulation of hypogastric nerve, pelvic nerves, colonic-rectal nerves and urethra-urinary bladder nerves evoked single and multiple synaptic inputs in pelvic plexus neurons. **Single synaptic inputs** are defined as either a single fast excitatory postsynaptic potentials (f-EPSPs) or a single action potential. **Multiple synaptic inputs** are defined as either a single action potential and a single f-EPSP and/or multiple f-EPSPs and/or multiple action potentials.

Figure 9b shows that > 55% of neurons received multiple synaptic inputs from all nerve trunks stimulated (75.0%, 153 of 205 from hypogastric nerve; 57.0%, 58 of 102 from pelvic nerves; 55.0%, 59 of 108 from colonic-rectal nerves and 59.0%, 64 of 109 from urethra-urinary bladder nerves). The remainder received single synaptic input (25.0%, 52 of 205 from hypogastric nerve; 43.0%, 44 of 102 from pelvic nerves; 45.0%, 49 of 108 from colonic-rectal nerves and 41.0%, 45 of 109 from urethra-urinary bladder nerves). The majority of neurons received multiple synaptic potentials, suggesting differences in the conduction velocity of the nerve fibers.

Figure 9. a: Synaptic inputs to neurons in pelvic plexus ganglia. Height of columns represents percentage of cells tested which received synaptic input from indicated nerve trunks. Numbers represent population of neurons tested.

b: Single and multiple synaptic inputs to neurons in pelvic plexus ganglia. Height of columns represents percentage of cells tested which received either single or multiple synaptic inputs from indicated nerve trunks. Single synaptic input (shaded column) consisted of either a single EPSP or a single action potential. Multiple synaptic inputs (open column) consisted of either a single action potential and a single EPSP and /or multiple EPSPs and /or multiple action potentials. Numbers represent the population of neurons which received synaptic response(s). HGN, hypogastric nerves; PELN, pelvic nerves; COL- RECN, colonic-rectal nerves and UUBN, urethra-urinary bladder nerves.


a
Percentage of Cells Tested
100
90
80
70
60
50
40
30
20
10
0
245
128
161
135
HGN
PELN
COL-RECN
UUBN


b
Percentage of Cells Tested
80
70
60
50
40
30
20
10
0
205
102
108
109
HGN
PELN
COL-RECN
UUBN

Convergent synaptic inputs from central and peripheral nerve trunks to a single neuron in pelvic plexus ganglia. To study the convergent synaptic inputs from central and peripheral sources, electrical stimulation was used to activate hypogastric nerve, pelvic nerves, colonic-rectal nerves and urethra-urinary bladder nerves. The distribution of neurons that receive synaptic inputs (consisting of f-EPSPs and action potentials) from one, two, three and four nerve trunks was determined.

Convergent synaptic input was tested in 107 neurons. Figure 10a shows convergent synaptic input to one neuron in pelvic plexus ganglia during electrical stimulation of preganglionic fibers in colonic-rectal nerves, urethra-urinary bladder nerves, pelvic nerves and hypogastric nerve. The distribution of neurons that receive synaptic inputs (f-EPSPs and action potentials) from one, two, three or four nerve trunks is shown in Figure 10b. Eighty-two percent of neurons tested (88 of 107) received convergent synaptic input mediated by acetylcholine nicotinic receptors from fibers within two, three and four nerve trunks. Fifteen percent of neurons (16 of 107) received synaptic input from fibers within one nerve trunk. The remainder received no synaptic input from any of the nerve trunks.

The data indicate that the majority of neurons in pelvic plexus ganglia receive convergent synaptic inputs from fibers within the hypogastric nerve, pelvic nerves, colonic-rectal nerves and urethra-urinary bladder nerves.

Origin of synaptic potentials evoked by electrical stimulation of colonic-rectal nerves and urethra-urinary bladder nerves. To determine whether the synaptic inputs to neurons in pelvic plexus ganglia elicited by electrical stimulation of colonic-rectal and urethra-urinary bladder nerves were mediated *via* collaterals of primary afferent fibers and preganglionic or postganglionic efferent fibers in hypogastric and pelvic nerves, synaptic transmission was studied following degeneration of fibers in the hypogastric and pelvic nerves (decentralization).

Figure 10. Convergence of synaptic input(s) to a neuron in pelvic plexus ganglia from central (hypogastric nerve and pelvic nerve) and peripheral nerve trunks (colonic-rectal nerves and urethra-urinary bladder nerves).

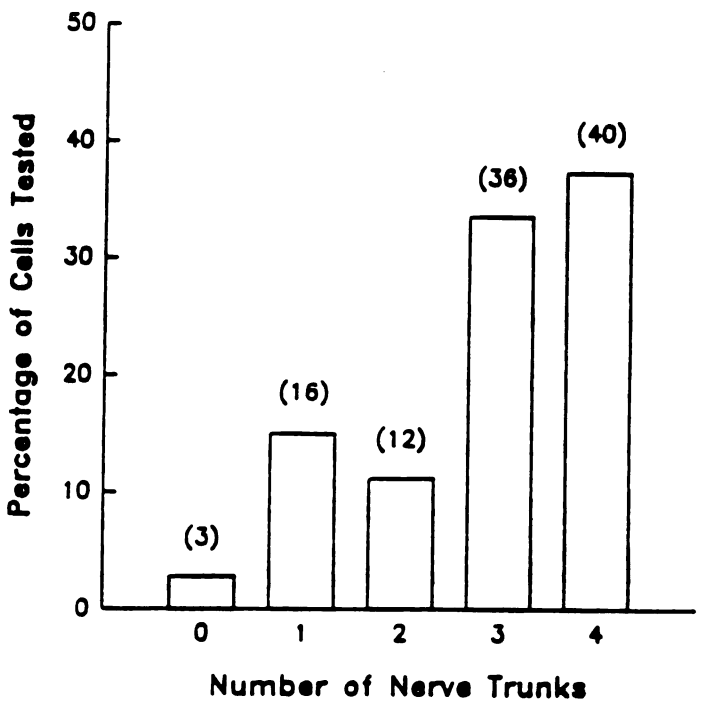
A: Convergent synaptic input to a single neuron in pelvic plexus ganglia when colonic-rectal nerve (a), pelvic nerve (b), urethra-urinary bladder (c) and hypogastric nerve (d) were electrically stimulated. Each panel represents superimposed traces of two successive responses to nerve stimulation at maximum intensity.

B: Height of columns represents the percentage of cells tested. The numbers (1-4) represent the number of convergent synaptic inputs to a single neuron. Note: 33% and 38% of neurons tested received convergent synaptic inputs from three or four nerve trunks, respectively. N=107, total number of neurons tested.

A


a
b
c
d
0
mv
-70
10 ms

B


Percentage of Cells Tested
50
40
30
20
10
0
(3)
(16)
(12)
(36)
(40)
0
1
2
3
4
Number of Nerve Trunks

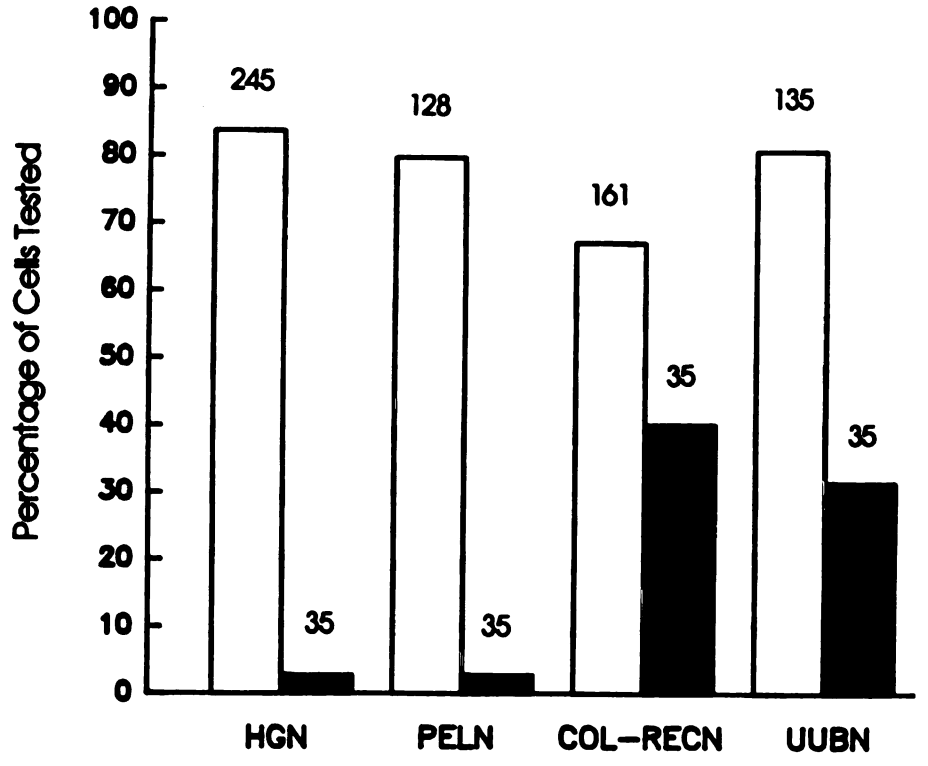


Figure 11. Synaptic input to pelvic plexus ganglion neurons in animals with an intact nervous system (open column) and after degeneration of preganglionic fibers in hypogastric nerves and pelvic nerves (dark column). The height of columns represents the percentage of cells tested which elicited excitatory synaptic potentials from the indicated nerve trunks at maximal stimulation intensities. HGN, hypogastric nerve. PELN, pelvic nerve. COL-RECN, colonic-rectal nerves. UUBN, urethra-urinary bladder nerves. Numbers on top of the columns represent the population of neurons tested.

The experiments were performed in 35 decentralized neurons of pelvic plexus ganglia from five guinea-pigs. Figure 11 shows the distribution of f-EPSPs and action potentials of neurons in animals with an intact nervous system (open column) and after degeneration of preganglionic fibers in hypogastric nerves and pelvic nerves (dark column). In animals for which fibers in hypogastric nerves and pelvic nerves had degenerated, the synaptic potentials elicited by electrical stimulation of hypogastric nerve and pelvic nerves were nearly abolished (2.8%, 1 of 35) while 40.0% (14 of 35) and 31.0% (11 of 35) of neurons tested received synaptic responses during electrical stimulation of colonic-rectal and urethra-urinary bladder nerves, respectively. The data suggest that approximately 30-40 % of synaptic responses originate from peripheral neurons located either in the myenteric plexus or submucous plexus of the distal colon or in intramural plexus of urethra-urinary bladder. The data also suggest that axon collateral fibers from afferent fibers and preganglionic efferent fibers in the hypogastric nerve and pelvic nerve may also provide synaptic inputs to neurons in pelvic plexus ganglia.

Table 3 shows the percentage of neurons that received single and multiple synaptic inputs during electrical stimulation of the respective nerve trunks. Data were obtained from animals with an intact nervous system and from animals after chronic degeneration of the hypogastric and pelvic nerves. Sixty percent of neurons in pelvic plexus ganglia received multiple synaptic inputs during electrical stimulation of the peripheral nerve trunks obtained from animals with an intact nervous system, while only 20-30 % of neurons in pelvic plexus ganglia received multiple synaptic inputs during electrical stimulation of the peripheral nerve trunks obtained from animals after degeneration of hypogastric and pelvic nerves. The data indicate that synaptic potentials recorded from neurons after chronic degeneration of hypogastric and pelvic nerves, consisted primarily of single f-EPSPs or single action potentials. The data in Table 4 indicate that the changes were not due to the alteration of active and passive electrical

Table 3. *Distribution of single and multiple synaptic potentials of PG neurons in animals with an intact nervous system and after degeneration of preganglionic fibers in hypogastric and pelvic nerves.*

	PG Neurons In Animals With An Intact Nervous System		PG Neurons In Animals With Preganglionic Fibers In HGN, PELN Degenerated	
Nerve Trunks Stimulated	Single Synaptic Potentials	Multiple Synaptic Potentials	Single Synaptic Potentials	Multiple Synaptic Potentials
COL-RECN	45.0% (49 of 108)	55.0% (59 of 108)	80.0% (26 of 31)	20.0% (5 of 31)
UUBN	41.0% (45 of 109)	59.0% (64 of 109)	73.0% (19 of 26)	27.0% (7 of 26)

COL-RECN, Colonic-rectal nerves; UUBN, Urethra-urinary bladder nerves.

Table 4. *Electrophysiological properties of neurons in normal, HGN transected and decentralized neurons in pelvic plexus ganglia of male guinea-pigs.*

Properties	Mean ± SE (N) Normal	HGN Degenerated	HGN + PELN Degenerated
Resting membrane potentials (mV)	-56.3 ± 0.3 (188)	-55.4 ± 1.1 (37)	-57.1 ± 1.3 (35)
Membrane input resistance (MΩ)	51.4 ± 2.5 (82)	49.7 ± 3.4 (18)	51.8 ± 3.4 (16)
Membrane time constant (τ, ms)	6.5 ± 0.3 (73)	5.3 ± 1.1 (18)	5.8 ± 0.9 (16)
Threshold current to initiate an AP (nA)	0.22 ± 0.03 (71)	0.15 ± 0.03 (18)	0.18 ± 0.03 (16)
Threshold potential to initiate an AP (mV)	12.1 ± 0.7 (71)	11.3 ± 1.0 (18)	12.0 ± 2.0 (16)
Amplitude of action potentials (mV)	73.0 ± 0.5 (179)	75.5 ± 4.7 (37)	74.2 ± 3.8 (35)
Action potential 50% duration (ms)	2.6 ± 0.1 (179)	2.3 ± 1.1 (37)	2.5 ± 1.3 (35)
ASH amplitude (mV)	13.9 ± 1.9 (78)	15.6 ± 0.8 (18)	13.0 ± 1.1 (16)
ASH 90% duration (ms)	126.9 ± 6.1 (78)	105.6 ± 13.9 (18)	143.2 ± 12.7 (16)

AP, action potential; ASH, after spike hyperpolarization; HGN, hypogastric nerve; PELN, pelvic nerves; (N), number of neurons tested.

properties of neurons, because there were no significant differences between parameters obtained from animals with an intact nervous system and from animals with degenerated hypogastric and pelvic nerves on these properties .

Conduction velocities of preganglionic fibers in central nerve trunks and fibers in peripheral nerve trunks. The calculated conduction velocities (m/s) of preganglionic fibers in hypogastric nerve, pelvic nerves and nerve fibers in colonic-rectal nerves and urethra-urinary bladder nerves were determined by measuring the latencies (ms) of synaptic responses during electrical stimulation of central and peripheral nerve fibers, subtracting 4.0 ms, a value estimating the ganglionic delay (Hartman and Krier, 1984) and dividing conduction distance (mm) by this value.

Fig. 12 shows the distribution of conduction velocities from fibers in hypogastric nerve, pelvic nerves, colonic-rectal nerves and urethra-urinary bladder nerves that provide synaptic inputs to neurons in pelvic plexus ganglia. Sixty % of fibers from all nerve trunks had conduction velocities ranging between 2 to 16 m/s. The remainder had the conduction velocities ranging between 0.1-2.0 m/s. The data indicate that the nerve fibers in peripheral nerve trunks and preganglionic fibers in central nerve trunks were B and C type fibers (Skok, 1979).

Antidromic Responses. Electrical stimulation of central (hypogastric and pelvic) and peripheral (colonic-rectal, urethra-urinary bladder) nerve trunks evoked all or none antidromic potentials in neurons of pelvic plexus ganglia. The potentials were resistant to the ganglionic blocking agent, hexamethonium (100 μM) or replaced by all or nothing fast rising potentials when nerve trunks are stimulated at high frequencies ranging between 20-50 Hz (Fig. 13). The percentage of neurons tested in pelvic plexus ganglia that exhibit antidromic potentials to electrical stimulation of central sympathetic (hypogastric), parasympathetic (pelvic) and peripheral (colonic-rectal, urethra-urinary bladder) nerve trunks is summarized in Fig. 14a. During stimulation of central nerve

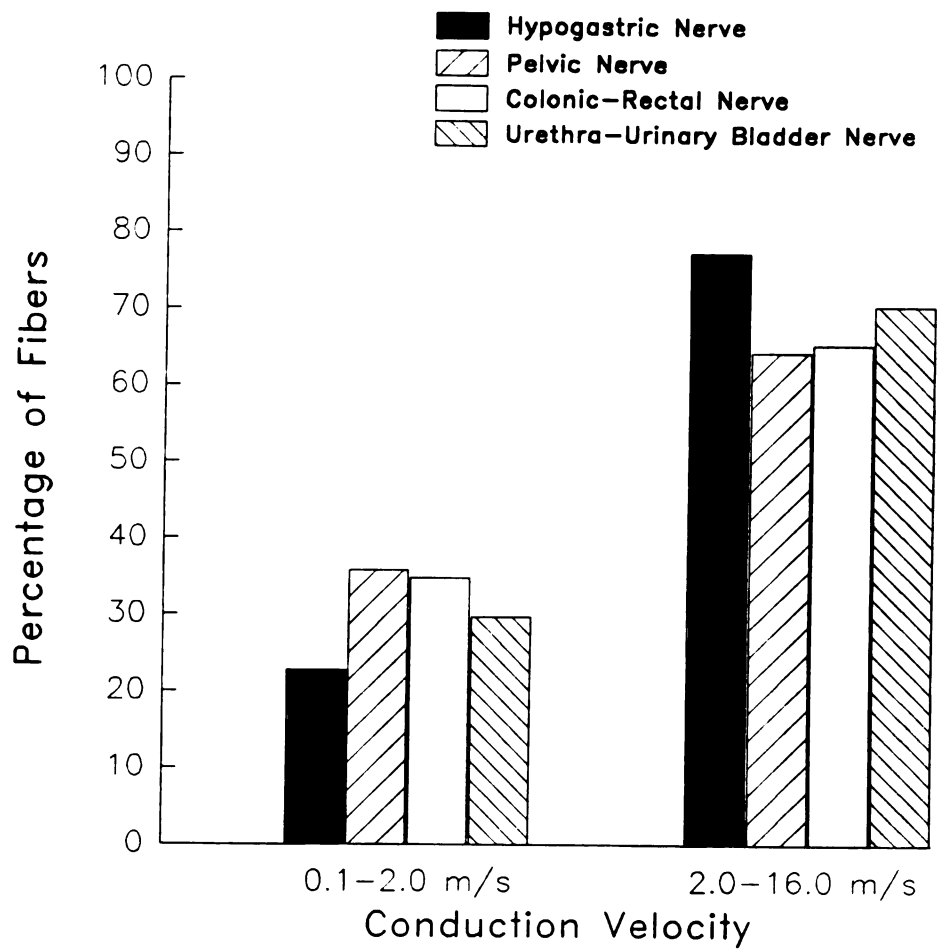


Figure 12. Calculated conduction velocities of preganglionic fibers which provide synaptic inputs to neurons in pelvic plexus ganglia. Height of columns represent percentage of fibers from indicated nerve trunks.

Figure 13. Antidromic action potential recorded from one neuron in pelvic plexus ganglia during electrical stimulation of urethra-urinary bladder nerves at 0.5 Hz (A), 15 Hz (C), 20 Hz (D) and 50 Hz (E) when superfused with a normal Krebs solution and at 0.5 Hz when superfused with a Krebs solution containing hexamethonium (10 μM) (B).

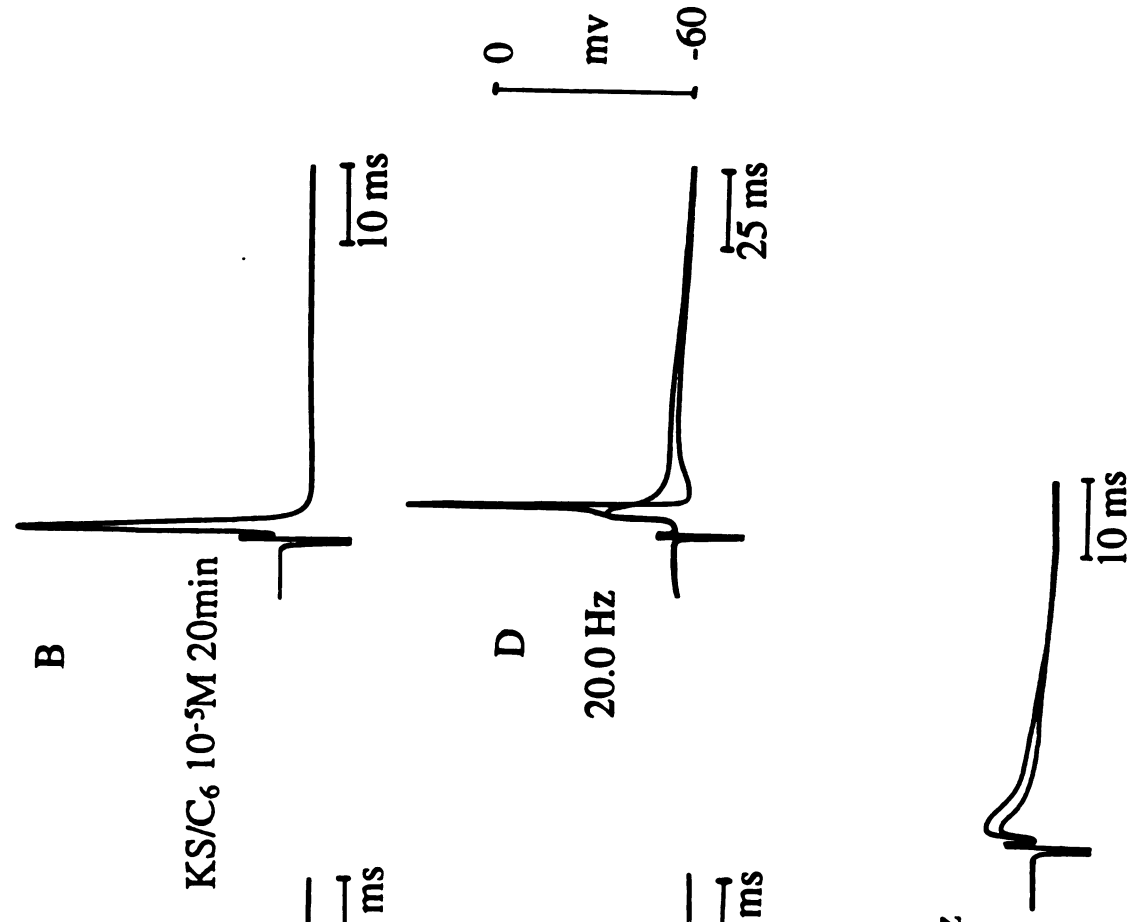
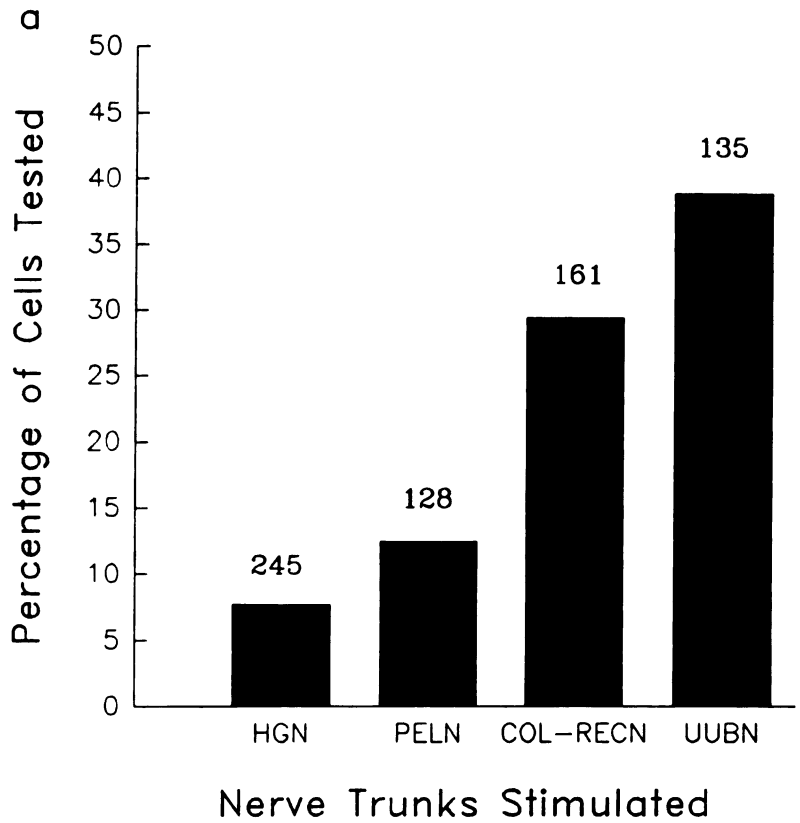
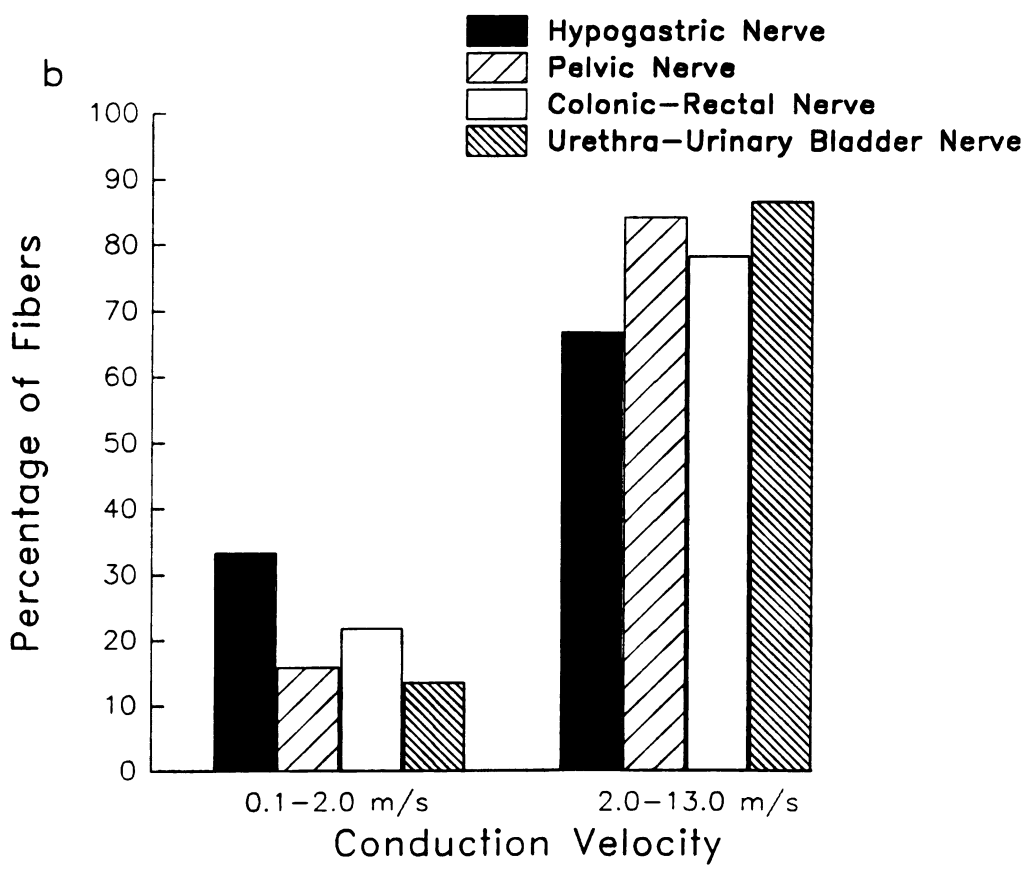

A
0.5 Hz
10 ms
B
KS/C6 10-5M 20min
10 ms
C
15.0 Hz
25 ms
D
20.0 Hz
25 ms
0
mv
-60
E
50.0 Hz
10 ms

Figure 14. a: Antidromic response to neurons in pelvic plexus ganglia. Height of columns represent percentage of cells tested which received antidromic response from indicated nerve trunks. Numbers represent population of neurons tested. HGN, hypogastric nerves; PELN, pelvic nerves; COL- RECN, colonic-rectal nerves and UUBN, urethra-urinary bladder nerves. b: Calculated conduction velocities of nerve fibers which provide antidromic inputs to neurons in pelvic plexus ganglia. Height of columns represent percentage of fibers from indicated nerve trunks.


a
Percentage of Cells Tested
50
45
40
35
30
25
20
15
10
5
0
245
128
161
135
HGN
PELN
COL–RECN
UUBN
Nerve Trunks Stimulated


b
Hypogastric Nerve
Pelvic Nerve
Colonic–Rectal Nerve
Urethra–Urinary Bladder Nerve
Percentage of Fibers
100
90
80
70
60
50
40
30
20
10
0
0.1–2.0 m/s
2.0–13.0 m/s
Conduction Velocity

trunks, about 8.0 % (19 of 245) of neurons tested responded with antidromic responses to electrical stimulation of hypogastric nerve, whereas 13.0 % (16 of 128) of neurons tested responded with antidromic responses to electrical stimulation of pelvic nerves. During peripheral nerve trunks stimulation, 30.0 % (48 of 161) and 39.0 % (52 of 135) of neurons tested responded with antidromic responses to electrical stimulation of colonic-rectal nerves and urethra-urinary bladder nerves respectively.

The calculated conduction velocities (m/s) of postganglionic fibers in colonic-rectal nerves, urethra-urinary bladder nerves, hypogastric nerve and pelvic nerves were determined by measuring the latencies (ms) of antidromic potentials during electrical stimulation of central and peripheral nerve fibers and dividing conduction distance (mm) by this value.

Fig. 14b shows the distribution of conduction velocities from fibers in hypogastric nerve, pelvic nerves, colonic-rectal nerves and urethra-urinary bladder nerves that provide antidromic inputs to neurons in pelvic plexus ganglia. The majority (70-90%) of fibers from all nerve trunks had conduction velocities ranging between 2 to 13 m/s. The remainder had conduction velocities ranging between 0.1-2.0 m/s. These data suggest that the majority of postganglionic fibers in peripheral nerve trunks and central nerve trunks were B fibers (Skok, 1979).

Discussion

Intracellular recording techniques *in vitro* have previously been used to study synaptic transmission from central preganglionic sympathetic (hypogastric) and parasympathetic (pelvic) nerve fibers to neurons in pelvic plexus ganglia of male guinea-pigs (Blackman, Crowcroft, Devine, Holman and Yonemura, 1969; Crowcroft and Szurszeski, 1971; McLachlan, 1977) and cat colonic ganglia (Krier and Hartman, 1984). The present data are similar to the results obtained in neurons of guinea-pig pelvic plexus ganglia by other researchers (Blackman, Crowcroft, Devine, Holman and Yonemura,

1969; Crowcroft and Szurszeski, 1971; McLachlan, 1977) and the results obtained in cat colonic ganglia mentioned above (Krier and Hartman, 1984). The synaptic responses consist of f-EPSPs and orthodromic action potentials and are mediated by cholinergic nicotinic receptors. However, in addition to synaptic inputs from central nerve trunks, the present study shows that neurons in pelvic plexus ganglia receive synaptic inputs from nerve fibers that connect pelvic plexus ganglia with peripheral organs (distal colon and urethra-urinary bladder). These synaptic responses are also mediated by cholinergic nicotinic receptors. The present study is the first to investigate synaptic transmission which originates from peripheral organs. In the absence of orthodromic stimulation, spontaneous f-EPSPs and action potentials are rarely seen. This observation is consistent with that stated by Blackman et al. (Blackman, Crowcroft, Devine, Holman and Yonemura, 1969).

There are two patterns of nerve stimulation-evoked synaptic responses from each nerve fiber tract. In the first pattern (less than 50%), the superthreshold response of the neuron is either a single action potential or a single excitatory postsynaptic potential. In this pattern, the neurons may receive synaptic input from a single nerve fiber or several nerve fibers with similar thresholds and conduction velocities. In the second pattern (55.0%-75.0%), the superthreshold response of neurons consists of multiple potentials with either a single action potential and multiple f-EPSPs or a single action potential and single EPSP or multiple action potentials and multiple f-EPSPs. In this pattern, the neurons likely receive synaptic inputs from multiple nerve fibers with different conduction velocities. Both single and multiple patterns of synaptic potentials in guinea-pig pelvic plexus ganglion neurons are similar to pelvic plexus ganglion neurons in other species and to other mammalian parasympathetic and sympathetic ganglia. However, the distribution of these two patterns in different ganglia is different. Generally, the majority of neurons in parasympathetic ganglia such as adult rat submandibular ganglia (Lichtman, 1977) and cat ciliary ganglia (Martin and Pilar, 1963) were considered to receive single

synaptic potentials while majority of neurons in sympathetic ganglia such as guinea pig and rabbit superior cervical ganglia (Perri, Sacchi and Casella, 1970; Wallis and North, 1978) and guinea-pig inferior mesenteric ganglia (Crowcroft and Szurszewski, 1971) receive multiple synaptic potentials. Yet, in the cat colonic ganglia (Krier and Hartman, 1984) which is a parasympathetic ganglia, the majority of neurons receive multiple synaptic potentials. Traditionally, due to their higher percentage of neurons receiving multiple synaptic input (Crowcroft, Holman and Szurszewski, 1971), sympathetic ganglia have been considered to be involved in more complicated integration function than parasympathetic ganglia. However, the present study demonstrates that pelvic plexus ganglia, a parasympathetic ganglia, had distribution of synaptic input similar to that of sympathetic ganglia, indicating the role pelvic plexus ganglia plays is more complicated than a simple relay station like other researchers predicted (Crowcroft, Holman and Szurszewski, 1971).

The majority of neurons in pelvic plexus ganglia (82.0 %) receive convergent synaptic inputs from two, three and four nerve fiber tracts that involve both central sympathetic, parasympathetic and peripheral nerves. Fifteen percent of neurons receive synaptic input from only one nerve trunk. The remainder receive no synaptic inputs. It is possible that neurons that receive a non-convergent synaptic input may function as simple relay stations with little capability of modulating synaptic input arising from either the central nervous system or peripheral organs. In contrast, neurons that receive a convergent synaptic inputs may be capable of integrating inputs from central sympathetic, parasympathetic and peripheral nerves. The data also indicate that neurons in pelvic plexus ganglia integrate inputs from both central and peripheral nerve trunks. Thus it can be suggested that interactions between the central nervous system and peripheral organs or interactions between two peripheral organs (colon-rectum and urethra-urinary bladder) can take place or be regulated at the pelvic plexus ganglia level.

Sixty percent of the neurons tested exhibit synaptic potentials during electrical stimulation of peripheral nerve trunks (colonic-rectal nerves and urethra-urinary bladder nerves). There are several possible explanations of this finding. First, axon collateral fibers in pre-and/or postganglionic axons in hypogastric nerve or pelvic nerve may provide synaptic innervation to neurons in pelvic plexus ganglia. Second, collaterals of primary afferent fibers may provide synaptic innervation to neurons in pelvic plexus ganglia. Third, fibers that provide synaptic inputs to neurons in pelvic plexus ganglia may originate from peripheral neurons located in enteric ganglia of distal colon and in intramural ganglia of urinary bladder. There have been a number of previous studies indicating that neurons in sympathetic prevertebral ganglia (inferior mesenteric and celiac ganglia) received mechanoreceptive peripheral synaptic input from the colon and small intestine (Crowcroft, Holman and Szurszewski, 1971; Kreulen and Szurszewski, 1979b). It is possible, therefore, that neurons in pelvic plexus ganglia receive a similar peripheral synaptic innervation. In chronic decentralized animals, the synaptic inputs from peripheral nerve trunks (colonic-rectal and urethra-urinary bladder nerves) are reduced, but still 30%- 40% of the synaptic responses remain. The results of the present study indicate that 30%-40% of the synaptic responses from electrical stimulation of peripheral nerves originate from peripheral organs. Also, we suggest that axon collateral fibers from afferent and pre/postganglionic fibers in hypogastric and pelvic nerves are involved. Eighty percent of the remaining responses are single responses with the majority consisting of single f-EPSPs. The passive and active electrical properties of neurons in pelvic plexus ganglia are not different. I conclude that the pattern of the synaptic potential change may due to the elimination of axon collaterals from central nerve fibers and not due to the changes in neuronal electrical properties.

From the conduction velocities, I conclud that the majority of preganglionic fibers in central nerve trunks are B and C fibers (conduction velocity of B fibers ranging from 2-15 m/sec; conduction velocity of C fibers ranging from 0.1-2.0 m/sec) (Skok, 1973).

The present data are similar to the data obtained for preganglionic fibers that synapse in sympathetic ganglia (inferior mesenteric ganglia, superior cervical ganglia and stellate ganglia) and parasympathetic ganglia (ciliary ganglia, intracardiac ganglia and submandibular ganglia) including guinea pig pelvic plexus ganglia (Skok, 1973). Also, the majority of the nerve fibers in peripheral nerve trunks that synapse with neurons in pelvic plexus ganglia are B and C fibers. The B and C fibers are generally afferent fibers which further supports the hypothesis that neurons in pelvic plexus ganglia may receive synaptic input from afferent fibers that originate from the periphery.

Antidromic potentials were recorded during electrical stimulation of central sympathetic (hypogastric) and parasympathetic (pelvic) nerves and peripheral nerve trunks (colonic-rectal and urethra-urinary bladder nerves). The results indicate that postganglionic neurons located in pelvic plexus ganglia send their axons both back to central nervous system and to periphery. Antidromic responses recorded from guinea-pig pelvic plexus ganglion neurons during electrical stimulation of the hypogastric nerve were also established in other laboratories (Blackman, Crowcroft, Devine, Holman and Yonemura, 1969). Our data also confirm the anatomy data obtained previously (McLachlan, 1985), where the application of horseradish peroxidase (HRP) to the distal end of the transected hypogastric nerve labels some neurons in pelvic plexus ganglia. Similar anatomical results were also obtained in the rats (Nadelhaft and Vera, 1991). It is not surprising that we found peripheral nerve trunks exhibited antidromic responses thus indicating that the pelvic plexus ganglion neurons send their postganglionic axons to distal colon, rectum, urethra-urinary bladder. It has previously been shown that postganglionic parasympathetic axons may provide phasic and tonic synaptic inputs to neurons in myenteric plexus or directly innervate colonic smooth muscle, blood vessels or mucus glands (de Groat and Krier, 1976; Hulter, Jodal and Lundgren, 1969; Wright, Florey and Jennings, 1938).

CHAPTER 3
NEURONS IN PELVIC PLEXUS GANGLIA RECEIVE MECHANOSENSORY INPUT FROM DISTAL COLON.

Introduction

Concept of Neurons in Prevertebral Ganglia Receiving Mechanosensory Input From Peripheral Organs.

The abdominal ganglia that lie anterior and anteriolateral to the abdominal aorta are called abdominal prevertebral ganglia. They are divided into four groups: celiac ganglia, superior mesenteric ganglia, inferior mesenteric ganglia and pelvic -hypogastric plexus. Neurons in the first three groups of prevertebral ganglia are mainly adrenergic while neurons in pelvic-hypogastric plexus are mainly cholinergic (Szurszewski and King, 1989)

The concept that neurons in guinea-pig prevertebral ganglia (inferior mesenteric, superior mesenteric ganglia and celiac plexus ganglia) receive excitatory cholinergic synaptic inputs consisting of f-EPSPs and action potentials originating from mechanoreceptors located in the peripheral organs has been demonstrated by using intracellular recording techniques and *in vitro* preparations which consist of an isolated segment of small intestine or distal colon attached to the prevertebral sympathetic ganglia (Crowcroft, Holman and Szurszewski, 1971; Weems and Szurszewski, 1977; Kreulen and Szurszewski, 1979a; Kreulen, Muir and Szurszewski, 1983). Furthermore, studies presented in Chapter 2, have shown that neurons in pelvic plexus ganglia also receive cholinergic synaptic inputs from fibers within peripheral nerve trunks that connect the pelvic plexus ganglia with effector organs such as distal colon or urethra-urinary bladder.

Properties of Visceral Mechanoreceptors in Distal Colon-Rectum

Two types of mechanoreceptors have been described in the gastrointestinal system (Iggo, 1957). One type, termed tension receptors, is located in serial with smooth muscle cells and exhibits a low threshold pressure to passive distension. Tension receptors are spontaneously active and their activities are increased by contractions of smooth muscle. The second type, termed stretch receptors, is located in parallel with smooth muscle cells and exhibits a high threshold for activation by passive distension. These mechanoreceptors are not spontaneously active and can not be activated by active contractions of the smooth muscle. They are activated by fast rates of passive distension and exhibit a rapidly adapting discharge of action potentials. The studies from human rectum showed that the mechanoreceptors located within the smooth muscle layers, exhibited properties of tension receptors while mechanoreceptors located on mucosa, exhibited properties of stretch mechanoreceptors (Sun et al., 1990). In guinea-pig distal colon, the mechanoreceptors that mediate synaptic inputs from colon to neurons in inferior mesenteric ganglia and from small intestine to neurons in celiac ganglia, inferior mesenteric ganglia have the properties of tension receptors (Szurszewski and King, 1989) and not stretch mechanoreceptors, because both distension and contraction of the colon elicit mechanosensory synaptic input (Kreulen and Peters, 1986). A stretch-activated mechanoreceptor would not be stimulated by a decrease in intestinal or colonic diameter during contraction.

Based upon the response of individual primary afferent fibers to increasing levels of balloon distension, mechanoreceptors in the gastrointestinal tract have been classified into three types. They are low threshold mechanoreceptors (LTM), high threshold mechanoreceptors (HTM) and wide-dynamic range mechanoreceptors (WDM) (Grundy, 1993).

Classification of mechanoreceptive primary afferents in cat colon-rectum.

Primary afferent fibers in cat colon-rectum have been classified on the basis of their size, conduction velocities and discharge of action potentials. One type are small diameter myelinated fibers with a median conduction velocity of 8.0 m/s. Their activation by mechanosensory stimuli causes a phasic discharge of action potentials. The other type are small diameter non-myelinated fibers with a median conduction velocity of 1.7 m/s. Their activation by mechanosensory stimuli causes a tonic discharge of action potentials (Jänig and Koltzenberg, 1991). Some nonmyelinated afferent fibers (C-fibers) are activated by mechanosenstive stimuli only after application of bradykinin, hypertonic KCl or by ischemia (Jänig and Koltzenberg, 1991; Haupt,. Jänig and Kohler, 1983) suggesting that these are silent C-fibers that are activated by mechanosensory stimuli during inflammation.

From the studies in last chapter, it has been shown that part of the synaptic input to neurons in pelvic plexus ganglia are purely of peripheral origin. We hypothesize that the peripheral synaptic inputs are mediated by mechanoreceptors located in the peripheral organ. The aim of the present study was to determine whether the synaptic inputs originating from peripheral organs are mediated by mechanoreceptors. The experiments were performed on a newly designed *in vitro* pelvic plexus ganglia-colon preparation. The data show that neurons in pelvic plexus ganglia receive nicotinic cholinergic synaptic inputs from distal colon. The synaptic inputs are mediated by mechanosensitive afferent fibers within the colonic-rectal nerves originated from the distal colon. Both tension and stretch receptors are involved.

Methods

Experiments were performed on adult male guinea-pigs (200-500 gm), euthanized by exsanguination following carbon dioxide gas-induced anesthetization. Pelvic plexus ganglia (PG), pelvic nerves, hypogastric nerves, urethra-urinary bladder nerves, colonic-

rectal nerves with an attached segment of distal colon-rectum were dissected *in situ* and placed in a two compartment organ bath (see Fig. 7). The distal colon (6-10 cm in length, originating 2 cm orad from the anus) was placed in one compartment of the organ bath and the PG and attached nerve trunks were placed in the other. The colonic nerves which connect the colon to PG were draped across a 1 mm thick wall separating the two compartments and covered by moist strips of tissue paper to prevent dehydration. Both bath compartments were superfused separately with a modified Krebs solution containing (in mM): 117 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.5 $NaHCO_3$ and 11 glucose. Solutions were gassed with 95% O_2-5% CO_2 and preheated to 37-38°C at the recording site. In all preparations, pelvic plexus ganglia were pinned onto Sylgard at the bottom of the chamber.

Measurement of intraluminal colonic pressure. The distal colon-rectum was cannulated at both ends for distension and measurement of intraluminal colonic/rectal pressure. The catheter at the orad end of the distal colon-rectum was connected to a pressure transducer to continuously monitor intraluminal pressure. The catheter at the aborad end was attached to a Y-valve with one end connected to a calibrated cylindrical reservoir and the other to a syringe. Basal intraluminal pressures (2-6 cm H_2O) were set by the height of Krebs solution in the calibrated reservoir. Colonic distension was performed by switching the position of the Y-valve from the reservoir to the syringe and injecting Krebs solution into the colon segment.

Electrophysiological procedures. Intracellular potentials were recorded with microelectrodes filled with 3 M- KCl. Tip resistance of the microelectrodes ranged between 60-110 MΩ. Membrane potential was recorded by conventional microelectrode techniques. An active bridge circuit will allow current injection into neurons through the recording microelectrodes. Signals from the microelectrode were displayed on an oscilloscope with digitized memory and recorded on a pen-writing chart recorder.

Both mechanical and electrical data (intraluminal pressure, individual electrotonic potentials, f-EPSPs and action potentials) were stored on a videocassette data recorder and were plotted on a pen-writing chart recorder.

Drugs were dissolved in Krebs solution and were superfused separately in either the colon-rectal compartment or the ganglia compartment of the organ bath.

Drugs used were hexamethonium bromide (C_6, Sigma), dihydro-β-erythroidine (Sigma), atropine sulfate (Sigma), tetrodotoxin (TTX, Sigma).

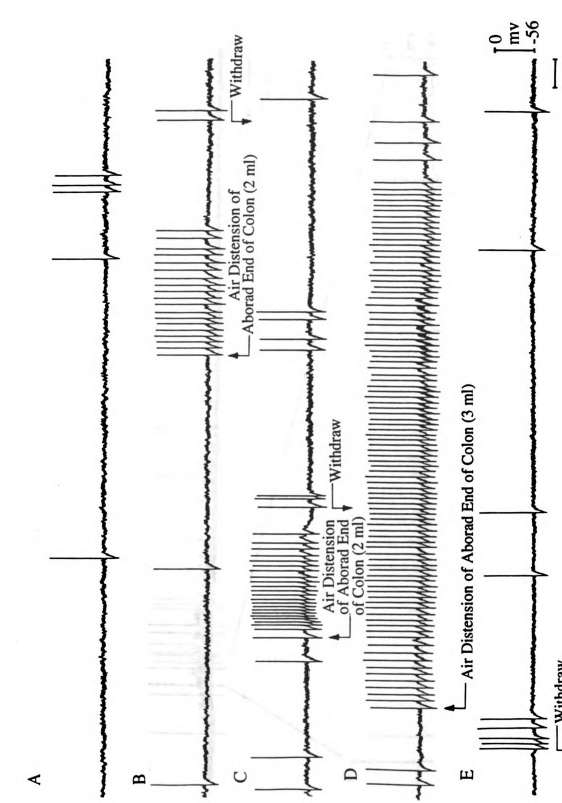
Data were expressed as mean ± SEM Student's *t* test was used for paired comparisons. $p< 0.05$ will be considered as significant.

Results

Mechanoreceptive synaptic inputs to neurons in pelvic plexus ganglia were studied in 202 neurons from pelvic plexus ganglia which were attached to a segment of distal colon-rectum via colonic-rectal nerves. Twenty-seven percent (54 of 202) of neurons exhibited continuous electrical activities consisting of either f-EPSPs (Fig. 15 panels A and B) or f-EPSPs and action potentials (APs) (Fig. 15, panels C, D, E and F). In 20% (11 of 54) of these neurons, the frequency of APs and f-EPSPs and amplitude of f-EPSPs were increased by distension of the colon segment (Fig. 16) or by applying punctate mechanoreceptive stimuli (using a glass rod with tip diameter of 2 mm) to the serosal/longitudinal muscle layer (data not shown). The responses showed adaptation even when the colon segment was still distended. The majority of neurons (73%, 148 of 202) in pelvic plexus ganglia were electrically silent. In 24% (35 of 148) of these neurons, passive colonic distension initiated f-EPSPs and APs that rapidly adapted (compare to the responses in neurons with continuous electrical activities) during the distending stimulus (Fig. 17). Slow synaptic responses evoked by distal colon distension were not observed. The properties and distributions of these two types of mechanosensitive responses are summarized in Table 5.

Figure 15. Excitatory synaptic inputs to three neurons (A and B; C and D; E and F) in pelvic plexus ganglia. Pelvic plexus ganglia were attached to distal colon-rectum via colonic-rectal nerves.

Figure 16. Effect of distension of distal colon on synaptic input to a neuron in pelvic plexus ganglia. Panel B-E show effect of distension and withdraw of air from distal colon.


A
B
C
D
E
Air Distension of Aborad End of Colon (2 ml)
Withdraw
Air Distension of Aborad End of Colon (2 ml)
Withdraw
Air Distension of Aborad End of Colon (3 ml)
Withdraw
0
mv
-.56
10 sec.

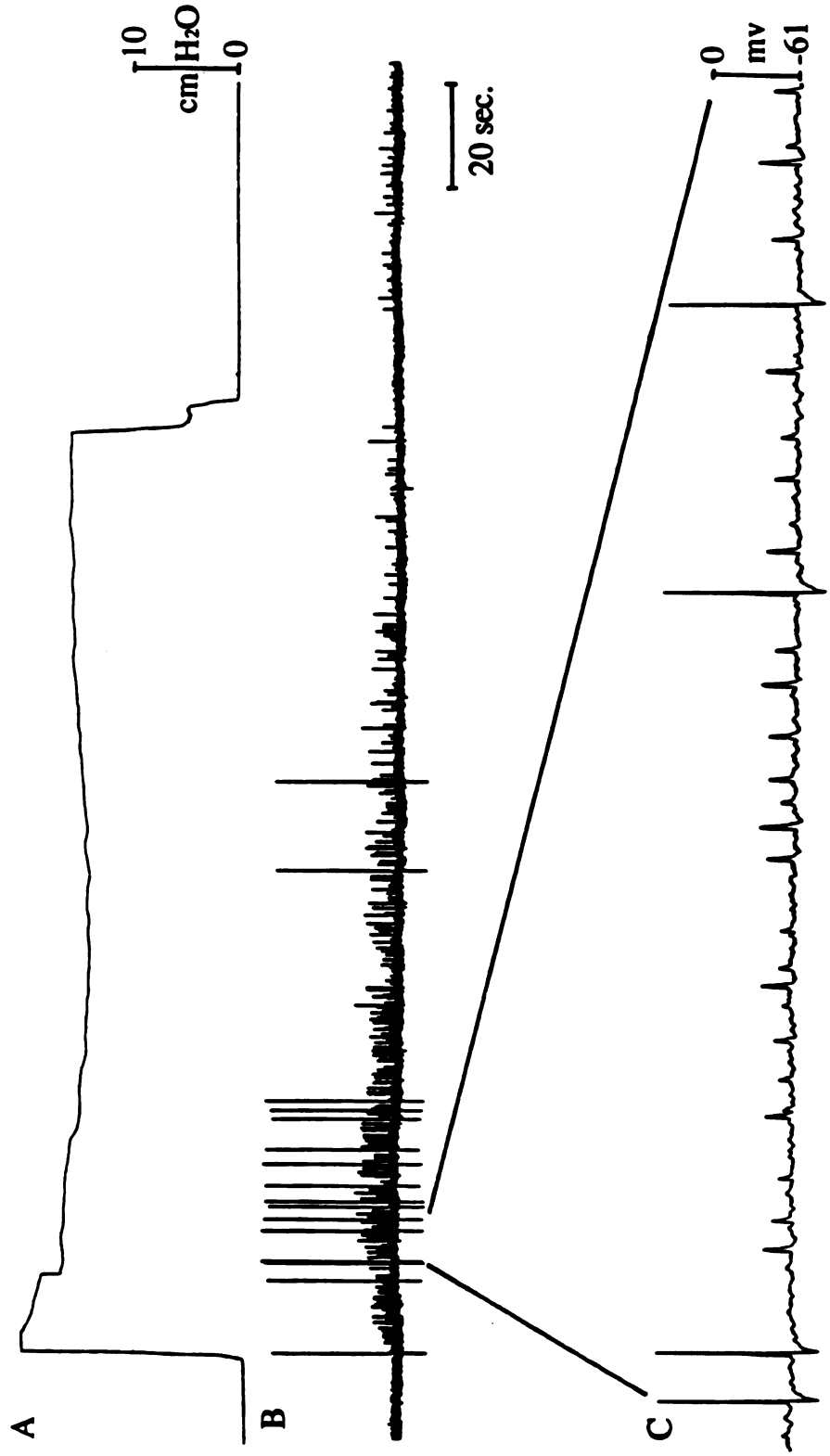
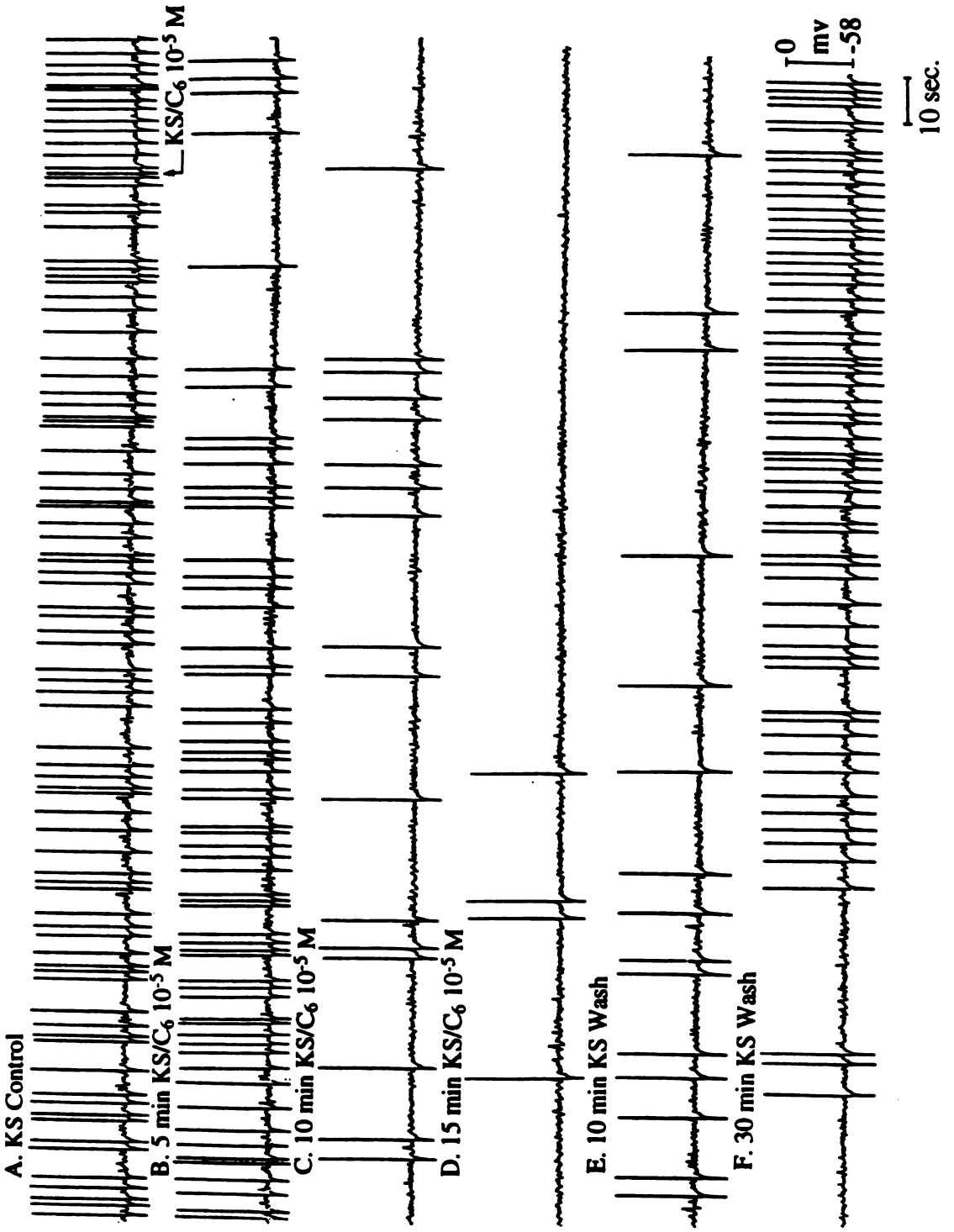


Figure 17. Effect of distal colon distension on initiating synaptic responses to a quiescent neuron in pelvic plexus ganglia. A, intraluminal colonic pressure. B, intracellular recording from a neuron in pelvic plexus ganglia. C, expanded trace from B indicated. Note: synaptic responses consisted of excitatory postsynaptic potentials and action potentials.

Figure 18. Effect of hexamethonium (C_6) (10 μM) on synaptic inputs from distal colon to a neuron in pelvic plexus ganglia. A, synaptic responses of a neuron in normal Krebs solution (KS). B, C and D, synaptic inputs to the same neuron 5, 10 and 15 minutes after superfusion of ganglia with a Krebs-hexamethonium solution (KS/C_6, 10 μM). E and F, recovery of synaptic responses when solution bathing the pelvic plexus ganglia was returned to normal Krebs solution 10 minutes and 30 minutes, respectively.


A. KS Control
B. 5 min KS/C6 10-5 M
KS/C6 10-5 M
C. 10 min KS/C6 10-5 M
D. 15 min KS/C6 10-5 M
E. 10 min KS Wash
F. 30 min KS Wash
0
mv
-58
10 sec.

The spontaneous electrical activities were reversibly blocked (n=7) by superfusion of the ganglia compartment of the organ bath with a Krebs solution containing hexamethonium (C_6, 10 - 100 μM) or dihydro-β-erythroidine (10 -50 μM) (Fig. 18). The data suggest that nicotinic acetylcholine receptors on neurons in pelvic plexus ganglia were involved in maintaining those spontaneous electrical activities. Also, transaction of colonic-rectal nerve trunks which connect the colon-rectum with pelvic plexus ganglia irreversibly abolished spontaneous electrical activities of neurons in pelvic plexus ganglia, indicating that nerve fibers in colonic-rectal nerves mediated the electrical activities (n=4) (Fig. 19).

The properties of mechanoreceptive responses evoked in electrically silent neurons of pelvic plexus ganglia are analyzed in Figure 20 and 21 and summarized in Table 5. Figure 20a shows a plot of the relation between instantaneous firing frequency (1/interval between successive action potentials) and action potential intervals at a constant distention rate and various constant distension pressures. Figure 20b shows a plot of the relation between instantaneous firing frequency and action potential intervals at a constant distension pressure and various constant distension rates. Rate of distension was calculated as the ratio between the magnitude of initial maximum intraluminal pressure and the time period needed to reach the maximum intraluminal pressure. The data show that rapid adaptation appeared within 1/3 of the total response period. Figure 21 shows that neurons exhibited higher maximum instantaneous firing frequencies at higher intraluminal distending pressure when the distending rate was constant or at faster rate of distension when distending pressure was constant. For both spontaneously active and quiescent neurons, the relationship between maximum instantaneous firing frequency and pressure of distension at a constant distension rate (40 cm H_2O/sec) is illustrated in Figure 22. The data show that compared to quiescent neurons, spontaneously active neurons were further activated to reach the maximum response at a lower distension

pressure. Also, mechanosensitive stimuli evoked higher maximal instantaneous firing frequency in spontaneously active neurons than in quiescent neurons.

Figure 19. Effects of sectioning colonic-rectal nerves on synaptic inputs to a neuron in pelvic plexus ganglia. Pelvic plexus ganglia were attached to distal colon-rectum via colonic-rectal nerves. A, B and C, synaptic inputs before sectioning colonic-rectal nerves. C and D, effect of sectioning some colonic-rectal nerves. E and F, effect of sectioning additional colonic-rectal nerves.

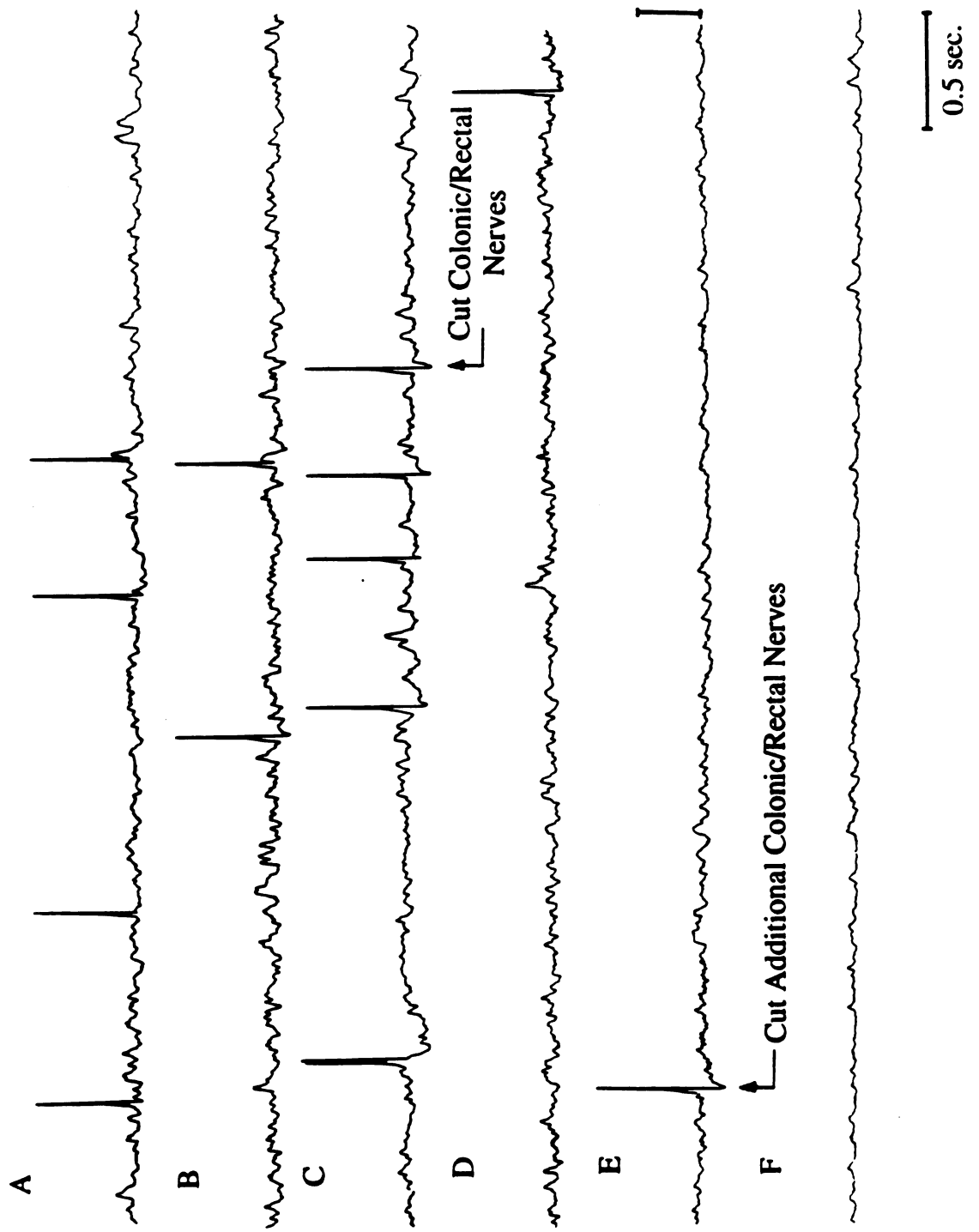

A
B
C
D
E
F
Cut Colonic/Rectal Nerves
Cut Additional Colonic/Rectal Nerves
0 mv -55
0.5 sec.

Figure 20. A: Plot of relation between instantaneous action potential frequency and action potential interval in four pelvic plexus ganglion neurons at a distension rate of 20 cm H_2O/sec (duration, 3 min). Distension pressures: 40 cm H_2O (▼-▼); 20 cm H_2O (∇-∇); 10 cm H_2O (● - ●); 6 cm H_2O (○ - ○). B: Plot of relation between instantaneous firing frequency and action potential intervals in five pelvic plexus ganglion neurons at a distension pressure of 40 cm H_2O.(duration, 3 min). Distension rates: 100 cm H_2O/sec (● - ●); 75 cm H_2O/sec (○ - ○); 60 cm H_2O/sec (∇ - ∇); 25 cm H_2O/sec (▼- ▼); 5 cm H_2O/sec (□ - □). Ordinate, instantaneous firing frequency. Abscissa, action potential interval.

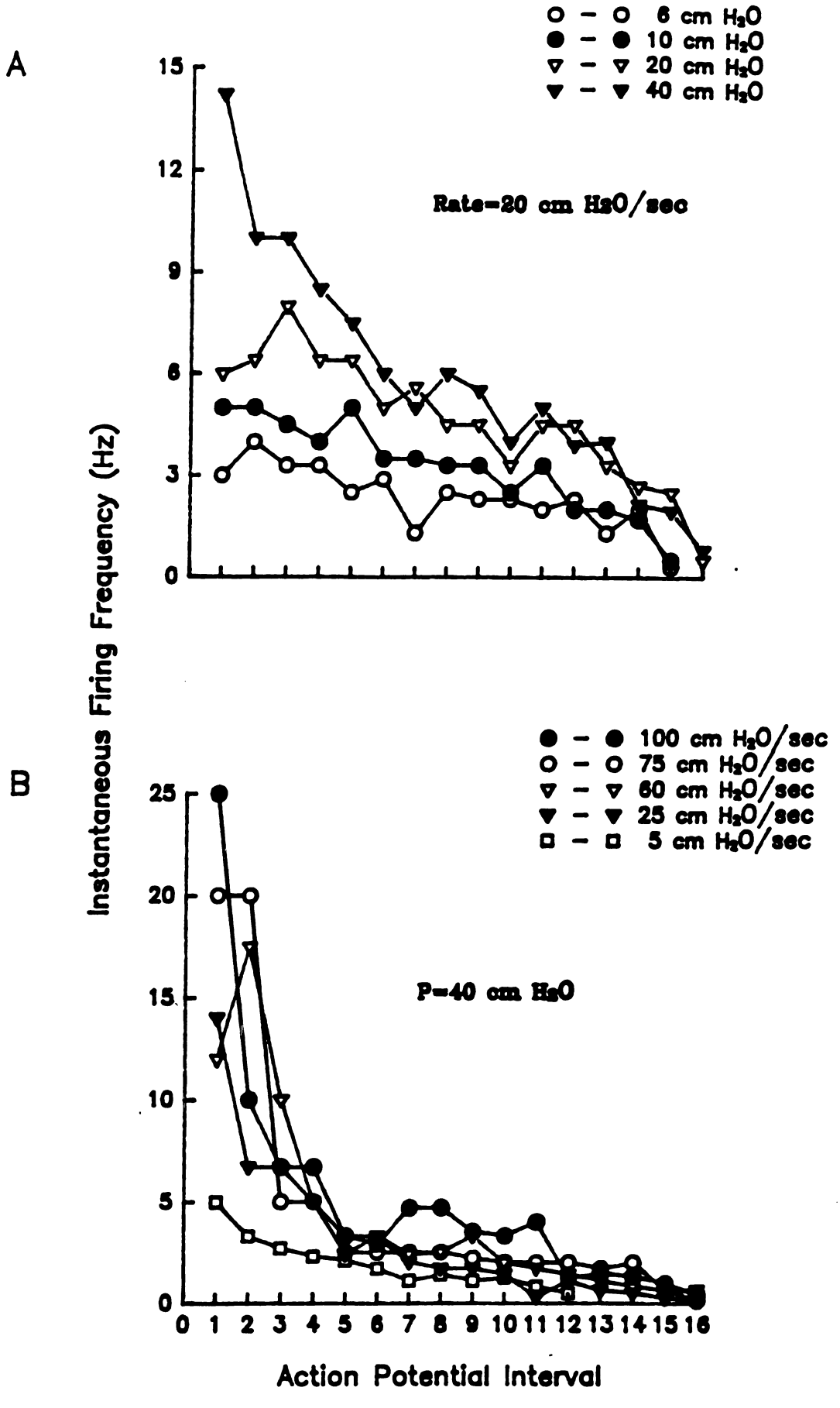
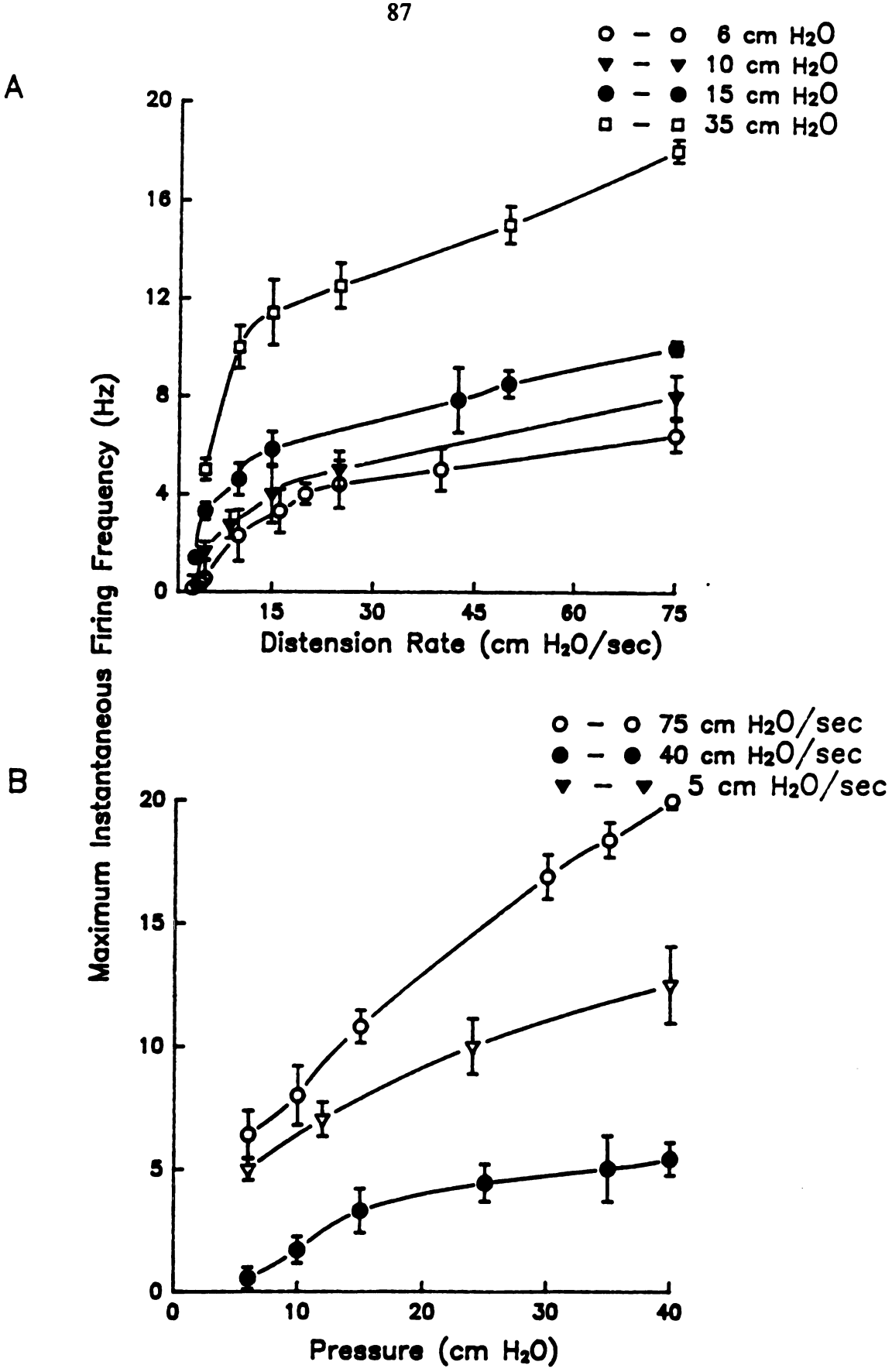

A
○ – ○ 6 cm H₂O
● – ● 10 cm H₂O
▽ – ▽ 20 cm H₂O
▼ – ▼ 40 cm H₂O
Rate=20 cm H₂O/sec
15
12
9
6
3
0
B
● – ● 100 cm H₂O/sec
○ – ○ 75 cm H₂O/sec
▽ – ▽ 60 cm H₂O/sec
▼ – ▼ 25 cm H₂O/sec
□ – □ 5 cm H₂O/sec
P=40 cm H₂O
25
20
15
10
5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Instantaneous Firing Frequency (Hz)
Action Potential Interval

Figure 21. Stimulus-response curves for distal colon mechanoreceptors. A: maximum instantaneous firing frequency plotted against distension rate at four different constant distension pressures. ❍ - ❍, 6 cm H_2O; ▼- ▼, 10 cm H_2O; ● - ●, 15 cm H_2O; □ - □, 35 cm H_2O. Ordinate, maximum instantaneous firing frequency. Abscissa, distension rate. B: maximum instantaneous firing frequency plotted against distension pressure at three different constant distension rates. ▼ - ▼, 5 cm H_2O/sec; ● - ●, 40 cm H_2O/sec; ❍ - ❍, 75 cm H_2O/sec. Ordinate, maximum instantaneous firing frequency. Abscissa, distension pressure.


A
o – o 6 cm H2O
▼ – ▼ 10 cm H2O
● – ● 15 cm H2O
□ – □ 35 cm H2O
Maximum Instantaneous Firing Frequency (Hz)
Distension Rate (cm H2O/sec)
B
o – o 75 cm H2O/sec
● – ● 40 cm H2O/sec
▼ – ▼ 5 cm H2O/sec
Pressure (cm H2O)

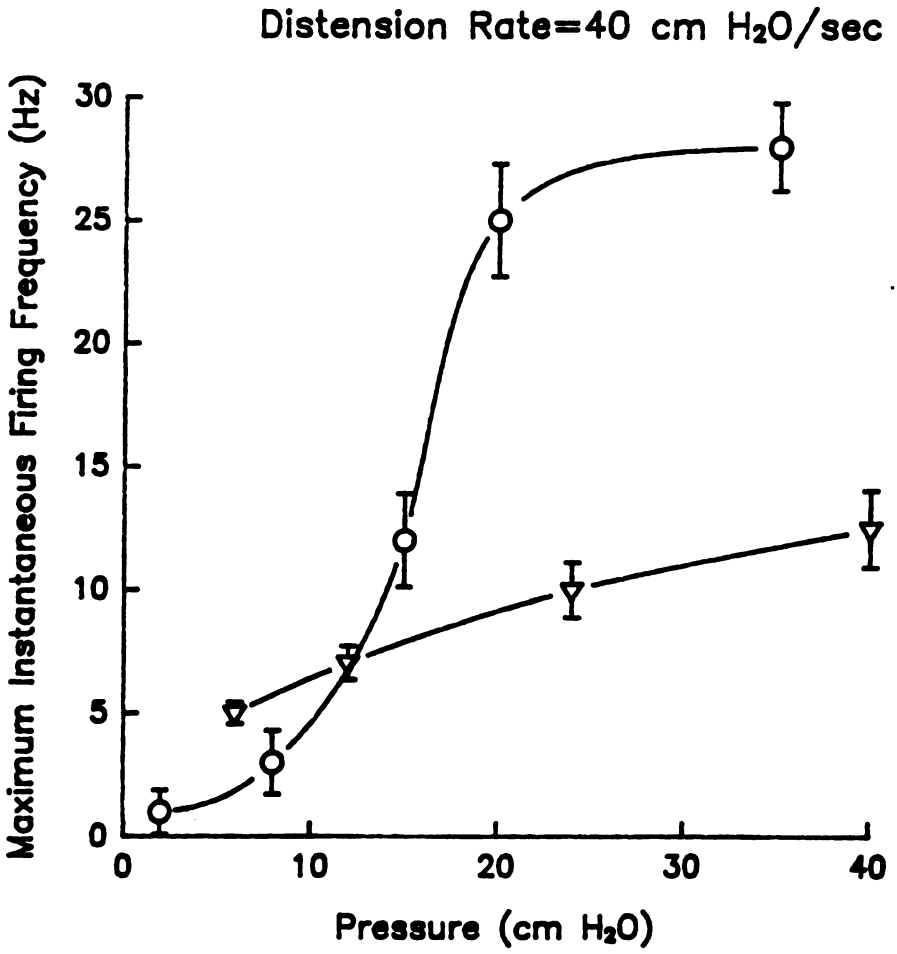


Figure 22. Stimulus-response curves for distal colon mechanoreceptors. Maximum instantaneous firing frequency is plotted against distension pressure for two types of mechanoreceptors mediated responses to spontaneous active (O-O) and quiescent (∇-∇) neurons in pelvic plexus ganglia. Ordinate, maximum instantaneous firing frequency. Abscissa, distension pressure.

Table 5. *Summary of mechanosensitive input(s) to neurons in pelvic plexus ganglia.*

Types of Neurons	Distribution	% Response to Mechanical Stimuli	Adaptation Time (sec.) Mean ± S.E.M.	Range	Possible Receptor Types Involved	Threshold of Activation (cm H_2O)
Spontaneous Active Neurons	27.0% (54 of 202)	20.0% (11 of 54)	48.5 ± 8.2	19.0 to 130.0	Tension and others	2.0 to 10.0
Quiescent Neurons	73.0% (148 of 202)	24.0% (35 of 148)	29.2 ± 9.9	3.0 to 43.0	Stretch	6.0 to 18.0

S.E.M., Standard Error of Mean.

Discussion

The present study shows that fast cholinergic nicotinic synaptic responses can be further activated or initiated by passive distal colon distension in a portion of neurons located in the pelvic plexus ganglia. The data demonstrate directly the existence of the afferent limb of pelvic plexus-pelvic organ reflexes involving neurons in pelvic plexus ganglia and mediated by mechanoreceptors located on the wall of the distal colon. Nearly 30% of neurons in the pelvic plexus ganglia exhibit continuous spontaneous electrical activity when a segment of distal colon is attached to the ganglia. In contrast, as shown in Chapters 1 and 2, spontaneous electrical activity was rarely seen in neurons of an isolated pelvic plexus ganglia. This can be considered as indirect evidence that continuous spontaneous electrical activity, recorded from neurons in pelvic plexus ganglia, is associated with a sensory input from the distal colon. Transecting the colonic-rectal nerves, which connect the distal colon-rectum with the pelvic plexus ganglia, abolishes the spontaneous electrical activity in neurons of pelvic plexus ganglia indicating directly that this activity originates from the distal colon-rectum. This concept is also supported by the fact that spontaneous activity of neurons in the pelvic plexus ganglia is reversibly blocked by nicotinic receptor antagonists, applied to the ganglia, suggesting that the electrical activities are due to synaptic input mediated by cholinergic nicotinic receptors.

Comparing the response of neurons in the pelvic plexus ganglia to the response of neurons in prevertebral sympathetic ganglia, several similarities and differences can be established. Similar to the spontaneously active neurons in the pelvic plexus ganglia, spontaneously active neurons in the prevertebral sympathetic ganglia receive mechanosensitive inputs mediated by tension mechanoreceptors. The tension mechanoreceptors providing synaptic input to neurons in the sympathetic ganglia can be activated by spontaneous contractions of the smooth muscle layers and exhibit low

thresholds to passive distension and no adaptation (Szurszewski and Weems, 1976; Kreulen and Szurszewski, 1979; Szurszewski and King, 1989). In contrast, the spontaneously active neurons in pelvic plexus ganglia, respond to passive distension of the distal colon with tonic discharge of action potentials and exhibit rapid adaptation. It is possible that continuous spontaneous electrical activity recorded from neurons in pelvic plexus ganglia is due to the activation of tension mechanoreceptors, while the increase in electrical activity, evoked by passive distension, represents the summarized response to activation of both tension and stretch mechanoreceptors. Stretch receptors are characterized as not spontaneously active, not activated by active contractions of the smooth muscle and exhibit relatively higher threshold, rapidly adapting discharge of action potentials (Iggo, 1957). This may explain why spontaneously active neurons show tonic discharge and adaptation to passive colon distension. The involvement of mixed mechanoreceptors, both tension as well as stretch receptors can be suggested. Both tension and stretch receptors have been shown to exist in the gastrointestinal system (Iggo, 1957; Sengupta and Gebhart, 1994).

The majority of neurons in pelvic plexus ganglia are quiescent and a portion of them are activated by rapid colon distension. This type of synaptic response rapidly adapts and correlates positively with both the rate of distension and pressure of distension. Stretch receptors but not tension receptors are considered to be involved. Based upon the response of individual primary afferent fibers to increasing levels of balloon distension, mechanoreceptors in the gastrointestinal tract have been classified into three types: low threshold mechanoreceptors (LTM), high threshold mechanoreceptors (HTM) and wide-dynamic range mechanoreceptors (WDM) (Grundy, 1993). Comparing the stimulus-response curves for esophageal mechanoreceptors (discharge frequency *vs.* distension pressure) (Grundy, 1993) to the data for the distal colon mechanoreceptors shown in the present study (Fig. 22), it can be suggested that both LTM and WDM are involved in the mechanosensory afferent inputs to

spontaneously active neurons and quiescent neurons in pelvic plexus ganglia, respectively.

Type B mechanosensitive sacral afferents (8 mm/sec) exhibit phasic responses to distension and type C (1.7 mm/sec) mechanosensitive sacral afferents exhibit a tonic response to distension. From the data presented in Chapter 2, nerve fibers that mediate synaptic inputs to neurons in pelvic plexus ganglia are type B and C nerve fibers. This is consistent with the present study where both tonic and phasic responses to distal colon distension were observed. The data suggest that mechanosensitive synaptic inputs to neurons in pelvic plexus ganglia are mediated by both type B and type C afferent fibers. Whether the type B and type C nerve fibers in peripheral nerve trunks are the same type B and type C fibers in the sacral afferents needs to be further elucidated. However, studies in myenteric neurons (Wood, 1989) have shown that two types of mechanosensitive units behave like visceral mechanoreceptors that discharge in primary afferents. One has the properties of fast-adapting (phasic) mechanoreceptors and the other behaves like slowly adapting (tonic) receptors

Unlike inferior mesenteric ganglia, where the majority (70%) of neurons receive mechanoreceptor mediated synaptic inputs from distal colon (Szurszewski and Weems, 1976), in pelvic plexus ganglia only a small percentage (25%) of neurons receive mechanosensitive synaptic inputs from distal colon. This may be due to either of the following possibilities: 1) neurons in pelvic plexus ganglia innervate multiple peripheral organs with only a small percentage of neurons innervating the distal colon; or 2). the majority of neurons in the pelvic plexus ganglia are efferent neurons. Neurons in inferior mesenteric ganglia receive both fast and slow synaptic potentials during colon distension. The fast synaptic potentials are mediated by nicotinic acetylcholine receptors, while the slow synaptic responses are noncholinergic. The distension-evoked noncholinergic slow synaptic responses are partially mediated by substance P (Kreulen and Peters, 1986; Peters and Kreulen, 1986). Substance P may be released from the axon collaterals of the

primary afferent fibers with cell bodies located in dorsal root ganglia or from substance P containing neurons located in myenteric plexus. In contrast, neurons in pelvic plexus ganglia respond to colon distension only with fast synaptic potentials mediated by nicotinic acetylcholine receptors indicating that only cholinergic afferents are involved.

CHAPTER 4

NEURONS IN PELVIC PLEXUS GANGLIA MEDIATE REFLEX CONTRACTIONS BETWEEN SEGMENTS OF COLON AND BETWEEN COLON AND URINARY BLADDER.

Introduction

Sacral Parasympathetic Reflex Pathways

Continence and defecation are the major functions of the colon and rectum under normal physiological conditions. These functions are regulated by extrinsic spinal visceral nerves (lumbar colonic and pelvic nerves) and local intrinsic nerve plexuses (myenteric and submucous plexus). In humans and experimental animals, the integrity of the parasympathetic pathways is essential for normal performance of defecation and continence. The importance of the sacral parasympathetic nerves has been shown in studies where pelvic nerves or sacral ventral roots were sectioned and the experimental animals were allowed to recover. The absence of this pathway, following chronic bilateral section of the pelvic nerves in cats, resulted in dilation of the colon, resembling megacolon and slowing of gastrointestinal transit (Adamson and Aird, 1932). In humans, destruction of the sacral ventral roots and pelvic nerves leads to fecal incontinence and to disturbances in defecation (Marzuk, 1985).

Data from physiological studies show that sacral parasympathetic fibers play an important role in colonic motility and defecation. Electrical stimulation of sacral ventral roots causes contractions of both longitudinal and circular muscle layers of the colon and rectum which often result in defecation. The studies performed in the cat by de Groat and Krier during the 1970s (de Groat and Krier, 1975, 1976 and 1978) brought a significant contribution to understanding the physiology of the large intestine. It was

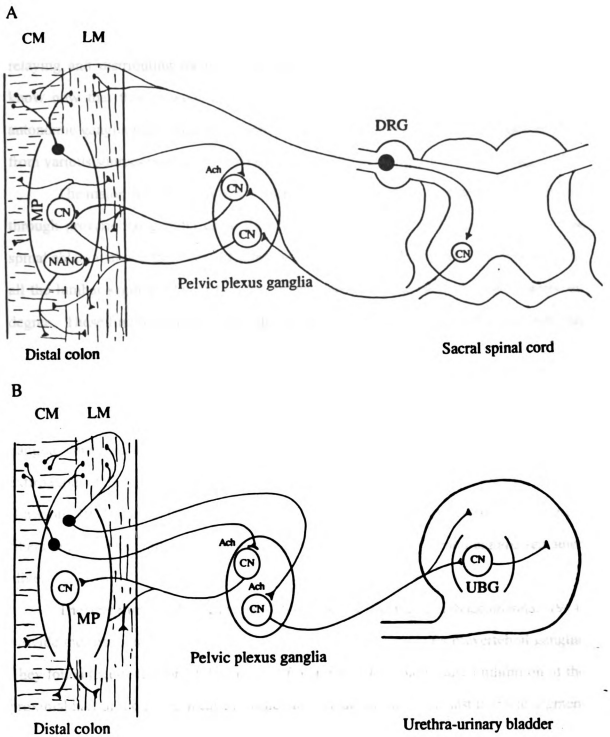
suggested that reflex activation of the sacral parasympathetic efferent pathway by distension, electrical stimulation or frictional stimulation leads to activation of primary afferent fibers and reflex firing in sacral parasympathetic efferent fibers. These reflexes enhance colonic motility leading to evacuation of colonic contents. The studies also demonstrated that the sacral parasympathetic reflexes are organized in the sacral spinal cord.

Based on the available experimental data, the organization of two possible sacral parasympathetic reflex pathways which can modulate colonic motility is illustrated in Figure 23. Figure 23a shows afferent fibers in colon circular (CM) and longitudinal muscle (LM) whose cell bodies are located in sacral dorsal root ganglia (DRG). They synapse with preganglionic efferent neurons in the sacral spinal cord. Preganglionic cholinergic neurons (CN) in the sacral spinal cord synapse with cholinergic neurons in the pelvic plexus ganglia. Neurons in the pelvic plexus synapse with the cholinergic (CN) and nonadrenergic, noncholinergic neurons (NANC) located in the myenteric plexus. Fibers from CN and NANC neurons in the myenteric plexus innervate CM and LM.

Sacral parasympathetic reflexes may also be organized in the pelvic plexus ganglia. Figure 23b shows afferent neurons (darkened circles) and their fibers in colon circular (CM) and longitudinal muscle (LM) that directly synapse with cholinergic neurons (CN) in pelvic plexus ganglia. The CN neurons synapse on smooth muscle and/or with cholinergic neurons located in the intramural plexus of the urinary bladder and the myenteric plexus of the colon. Fast excitatory cholinergic mechanosensory afferent inputs originating from distal colon have been demonstrated in neurons in pelvic plexus ganglia (see Chapter 3).

Figure 23. A: Diagrammatic sketch of sacral parasympathetic central reflex pathway to colon. The afferent fibers have their cell bodies located in sacral dorsal root ganglia, and terminals located in colon-rectum. The preganglionic fibers from the sacral spinal cord synapse either directly on colon smooth muscle and/or indirectly via myenteric plexus cholinergic (CN) or nonadrenergic, noncholinergic (NANC) neurons. CM, circular muscle; LM, longitudinal muscle; MP, myenteric plexus; DRG, dorsal root ganglia.

B: Diagrammatic sketch of sacral parasympathetic reflex pathway to colon and urinary bladder via pelvic plexus ganglia. The afferent neurons located in myenteric plexus and directly synapse with cholinergic neurons (CN) in pelvic plexus ganglia. These cholinergic neurons synapse either directly on smooth muscle and/or indirectly mediated by intrinsic plexus neurons of the urinary bladder or myenteric plexus neurons of colon. CM, circular muscle; LM, longitudinal muscle; MP, myenteric plexus;


A
CM
LM
DRG
Ach
CN
MP
CN
CN
NANC
CN
Pelvic plexus ganglia
Distal colon
Sacral spinal cord
B
CM
LM
Ach
CN
CN
Ach
MP
CN
UBG
Pelvic plexus ganglia
Distal colon
Urethra-urinary bladder

Peripheral Reflexes Mediated by Prevertebral Ganglia.

Before the late 1960s, prevertebral ganglia were viewed primarily as stations relaying and distributing incoming central instructions to peripheral destinations. We know now that this viewpoint is incomplete. In mammals, it has been shown that autonomic ganglia participate in peripheral reflexes, integrate synaptic messages arriving from various sources and utilize several neurotransmitters (Szurszewski and King, 1989).

The major impetus for renewed interest in the concept of peripheral reflex activity through prevertebral ganglia came from Garry (Garry, 1933, 1934), who showed that spinal ablation above the level of L_1 and below L_5, followed by dorsal root rhizotomy of all the lumbar segments failed to alter colonic movements in the cat to any significant degree. These findings cast doubt on the involvement of the spinal cord as the principal site of communication for afferent traffic. It is important to point out that Garry did not open the abdominal cavity and that his results reflected the activity of a normal, dynamic gut. In these experiments, Garry also showed that removing the inferior mesenteric ganglia considerably increased colonic motility. Subsequently, it was shown that extirpation of the inferior mesenteric ganglia in dogs also increased colonic motility (Lawson, 1934; Lawson and Holt, 1937). Thus the possible involvement of afferent fibers in peripheral reflexes synapsing on ganglion cells in prevertebral ganglia remained alive.

The experiments by Kuntz and Saccomanna (Kuntz and Saccomanna, 1944) provide the first concrete evidence for peripheral reflexes through a prevertebral ganglia. They found that distension of the distal half of a transacted colon caused inhibition of the proximal half of the colon in cats, in which the spinal cord from the last thoracic segment onward was removed, the lumbar sympathetic chain was removed from both sides, the intermesenteric and hypogastric nerves were cut, and the colon was transected at its midregion. Semba (Semba, 1954) also showed that an inhibitory intesto-intestinal reflex

did not depend on the continuity of intrinsic nerve plexuses in the gut nor on the spinal cord but depended on the integrity of the celiac plexus.

Inhibitory colon-colonic and intesto-intestinal reflexes, which are mediated by mechanoreceptors located in myenteric plexus of the distal colon and the small intestine and by neurons located in prevertebral sympathetic ganglia (inferior mesenteric ganglia and celiac ganglia, respectively) have also been directly demonstrated in *in vitro* colon-inferior mesenteric ganglia or small intestine-celiac ganglia preparations (Crowcroft, Holman and Szurszewski, 1971; Kreulen and Szurszewski, 1979b).

Effects of capsaicin on primary afferent fibers.

Capsaicin is the pungent ingredient in red peppers of the genus *Capsicum*. Chemically, it is a derivative of vanillyl amide, 8-methyl-N-vanillyl-6-nonenamide and has a molecular weight of 305.42. Högyes (1878) was the first to state that the pungent and irritant action of capsicol, an extract of capsaicin, is mainly mediated by sensory nerves. In 1960s, a Hungarian investigator, N. Jancsó, discovered that capsaicin, the pure substance, also exerts a long-term sensory receptor blocking action which can be used as an important probe to investigate the function of afferent neurons (Jancsó, 1960, 1968). The function of primary afferent neurons is to receive and transmit information from the internal and external environment and thereby contribute to the organism's ability to maintain homeostasis. The cell bodies of primary afferent neurons are located in the spinal cord (dorsal root) or cranial sensory ganglia and send fibers in both central and peripheral directions. They contain tachykinins (including substance P) and calcitonin gene related peptide (CGRP) (Holzer, 1988). The ending of the peripheral fibers may be either the receptors themselves or connected to special structures, providing the sensory information. Most of the current knowledge about primary afferent neurons is based on the application of new experimental techniques and capsaicin, which has proven to be an important pharmacological tool in sensory neuroscience.

Primary afferent fibers have been divided into three groups: (a) thick myelinated (Aα, β)-fibers have highest conduction velocities and carry non-nociceptive mechanical information from skin and muscle. (b) thin myelinated (Aδ)-fibers have intermediate velocities and carry both nociceptive (mechanonociceptors and polymodal nociceptors) and nonnociceptive (mechanoreceptors, cold receptors) and (c) thin myelinated (C)-fibers, have the slowest conduction velocities and are primarily nociceptors (polymodal nociceptors, chemonociceptors) which respond to noxious mechanical, thermal and/or chemical stimuli.

Acute capsaicin application depolarizes nonmyelinated (C)-fibers and small diameter myelinated sensory terminals (Aδ)-fibers (Heyman and Ran, 1985; Marsh et al., 1987; Bevan and Forbers, 1988). After the acute application of capsaicin to axons of sensory neurons, nerve conduction through the treated segment is blocked (Petsche et al., 1983; Handwerker et al., 1984; Brugger et al., 1990). Chronic administration of capsaicin to neonatal animals depletes tachykinins and CGRP from sensory fibers whose cell bodies are located in dorsal root ganglia and further destroys them (Buck and Burks, 1986). Also, systemic administration of capsaicin to adult guinea-pigs produces extensive axon terminal degeneration in the dorsal spinal cord (Jancsó, et al., 1987). Depletion of tachykinins and CGRP and ultrastructural signs of axon degeneration are appreciable for at least 1 year (Papka et al., 1984). In contrast, capsaicin does not deplete substance P and CGRP from enteric neurons in adult animals (Hoyes and Barber, 1981).

The mechanism for the action of capsaicin on primary afferent neurons and their axons has been established. Capsaicin-evoked depolarization involves an increase in cation conductance (Na^+ and Ca^{2+}) though a receptor-mediated mechanism (Marsh et al., 1988; Docherty, et al., 1991). Membrane depolarization causes further Na^+ and Ca^{2+} influx by activating voltage-dependent sodium and calcium channels (Wood et al., 1988). The increase in intracellular calcium due to calcium entry, secondarily causes a prolonged inhibition of voltage-activated calcium currents (Docherty et. al., 1991) and

initiates a neurotoxic process (Marsh et al., 1988; Jancsó et. al., 1984). Long lasting inhibition of voltage-activated calcium channels may contribute to the mechanism of capsaicin inhibition of neurotransmitter release from terminals of primary afferent neurons and neuronal toxicity may contribute to the degeneration of primary afferent neurons.

Previous studies have shown that acute capsaicin application *in vitro* can selectively "desensitize" primary sensory C fibers (Jancsó, 1977) and has been shown to abolish the primary afferent fiber mediated noncholinergic (second-phase) smooth muscle contraction in response to vagus nerve stimulation or electrical field stimulation (EFS) (Undem et al., 1990).

To test the involvement of axon collaterals from primary afferent fibers in reflex contractions between segments of colon and between colon and urinary bladder, acute capsaicin treatment at the site of pelvic plexus ganglia will be used. Capsaicin will deplete tachykinins and CGRP from the primary afferent fibers and eliminate the possible involvement of axon collaterals from primary afferent in the reflex contractions.

Neuropeptides in primary sensory neurons. It has been known that CGRP and takykinins are putative transmitters of sensory neurons. CGRP is a 37 amino acid peptide encoded in the calcitonin gene. Its expression is dependent on tissue-specific alternative RNA processing (Amara, Jonas, Rosenfeld, Ong and Evans, 1982; Rosenfeld, Mermod, Amara, Swanson, Sawchenko, Rivier, Vale and Evans, 1983). Immunohistochemical studies have revealed that CGRP is present throughout the central and peripheral nervous systems. The action of CGRP depends on the existence of specific CGRP receptors in target tissues. Using double-labeling techniques it was shown that CGRP and substance P coexist in primary afferent neurons. All substance P containing primary afferent neurons also contain CGRP, but there is also a population of nerve cells that contains only CGRP but not substance P (Lee, Takami, Kawai, Girgis, Hillyard, Macintype, Emson and Tohyama, 1985; Skofitsch and Jacobowitz, 1986;

Gibbins, Furness, Costa, MacIntyre and Girgis, 1985; Gibbins, Furness and Costa, 1987). The distribution of CGRP in the central and peripheral nervous system and its co-localization in some neurons with substance P or acetylcholine suggests several possible roles in autonomic sensory and motor functions as well as in inflammation (Goodman and Iversen, 1986; Sharkey, 1992). CGRP also has effects in the gastrointestinal system. In the guinea-pig, CGRP induced contraction of ileal and colonic longitudinal smooth muscle in a dose-dependent (10-200 nM) manner (Goodman and Iversen, 1986).

Chemically, the tachykinins (TKs) are a family of peptides that share the common C-terminal sequence, Phe-X-Gly-Leu-Met-NH_2.(Erspamer, 1981). In mammals, at least three different TKs namely substance P (SP), neurokinin A(NKA) and neurokinin B (NKB) are likely neurotransmitters both in central and peripheral nervous system (Maggi, 1990). Three neurokinin receptor subtypes (NK-1, NK-2 and NK-3) have been cloned, sequenced and pharmacologically characterized. Although each of the endogenous tachykinins can stimulate all of the receptors, there is a selectivity of SP, NKA and NKB for NK-1, NK-2 and NK-3 receptors, respectively (Maggi, 1990). NK-1 receptors are present on neurons, smooth muscle cells and neuroendocrine cells. NK-2 receptors occur primarily on smooth muscle cells while NK-3 receptors are located primarily on neurons (Buck and Burcher, 1986; Buck, Pruss, Krstenansky, Robinson and Stauderman, 1988; Yau and Youther, 1982). Tachykinin-induced contractile responses through the activation of tachykinin receptors have been shown in dog and guinea-pig ileum (Chahl, 1982; Shuttleworth, Sanders and Keef, 1993; Daniel, Parish, Watson, Fox-Threlkld, Regoli and Rainsford, 1995). SP- and NKB-induced release of acetylcholine has also been shown in guinea-pig small intestine (Yau and Youther, 1982; Yau, Mandel, Dorsett and Youther, 1992). In autonomic ganglia, SP and NKB induce membrane depolarization, as it was shown in bronchial parasympathetic ganglia (Myers and Undem, 1993). SP also induces membrane depolarization, associated with an increase in input

resistance in guinea-pig inferior mesenteric ganglia (Dun and Minota, 1981; Krier and Szurszewski, 1982).

Since neurons in pelvic plexus ganglia receive mechanoreceptor mediated synaptic inputs from peripheral organs as shown in Chapter 3, we hypothesize that neurons in pelvic plexus ganglia mediate visceral organ reflexes. The aim of the present section of the study is to determine whether pelvic plexus ganglia and nerve fibers which connect the pelvic plexus ganglia with peripheral organs (distal colon and urethra-urinary bladder) can mediate reflexes between colon segments and between colon and urethra-urinary bladder. We also aim to determine, if capsaicin-sensitive primary afferent axon collateral fibers are involved in these peripheral reflexes. The data suggest that neurons in pelvic plexus ganglia are able to mediate excitatory reflex responses between two isolated segment of distal colon and between distal colon and urinary bladder. These peripheral reflexes do not involve capsaicin sensitive axon collaterals from primary afferent fibers.

Methods

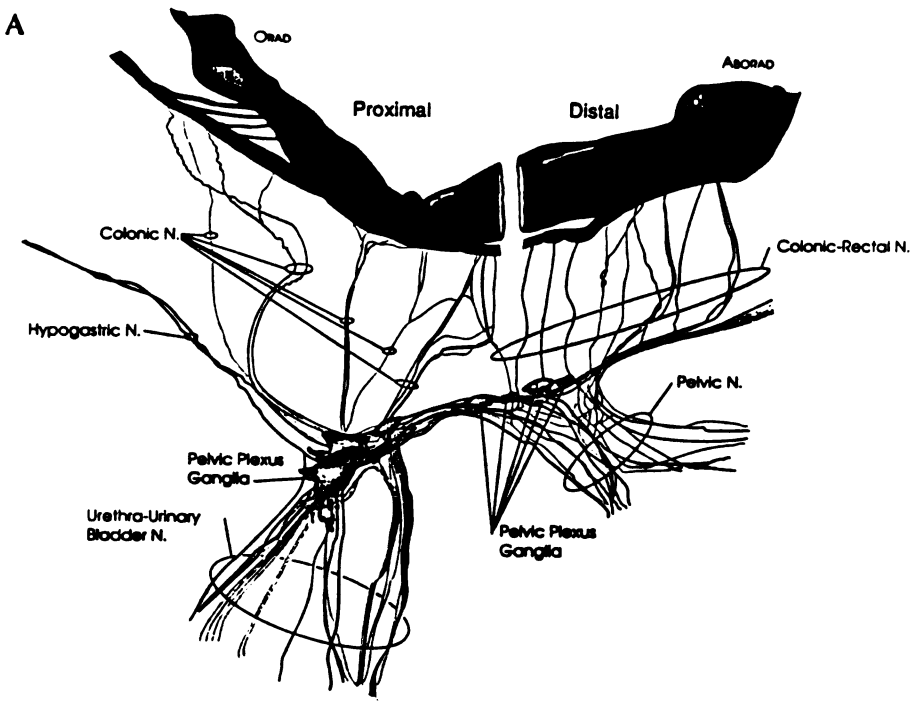
Experiments were performed on adult male guinea-pigs (200-500 gm), euthanized by exsanguination following carbon dioxide gas-induced anesthesia. Pelvic plexus ganglia (PG), pelvic nerves, hypogastric nerves, urethra-urinary bladder nerves, colonic-rectal nerves with an attached segment of distal colon-rectum and/or urethra-urinary bladder were dissected *in situ* and placed in a two or three compartment organ bath.

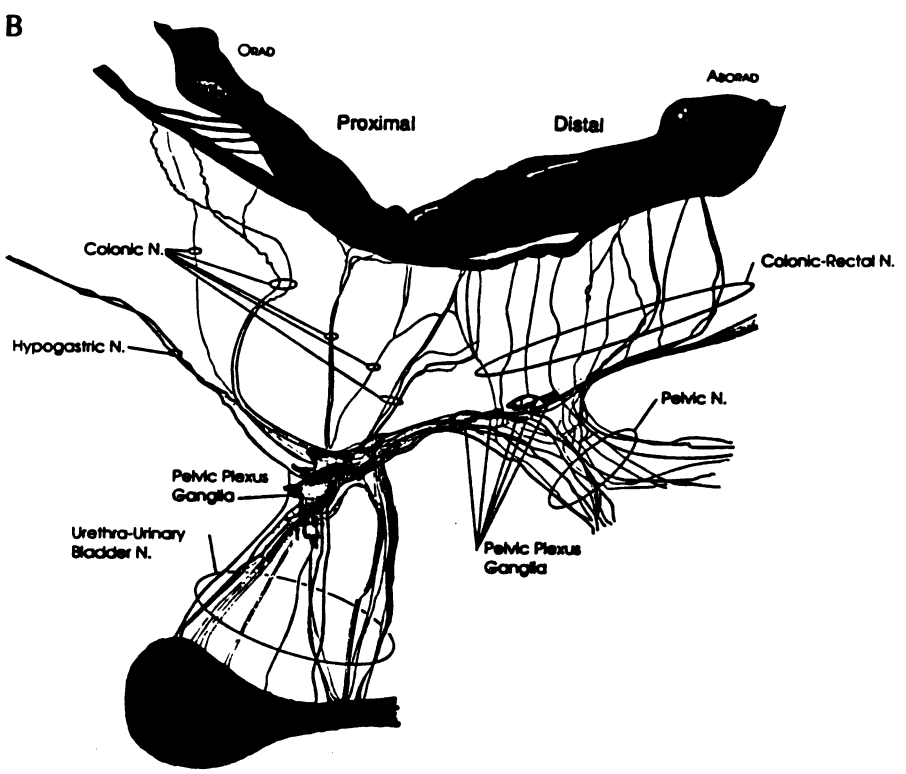
Measurement of intraluminal colonic pressure and urinary bladder pressure. The distal colon-rectum was canulated at both ends for distension and measurement of intraluminal colonic/rectal pressures. The catheter at the orad end of the distal colon-rectum was connected to a pressure transducer to continuously monitor intraluminal pressures. The catheter at the aborad end was attached to a Y-valve, with one end connected to a calibrated cylindrical reservoir and the other to a syringe. Basal

intraluminal pressures (2-6 cm H_2O) were set by the height of Krebs solution (KS) in the calibrated reservoir. Colonic distension was accomplished by switching the position of the Y-valve from the reservoir to the syringe and injecting Krebs solution into the colon segment. The urinary bladder was cannulated through the urethra for either distension or measurement of intraluminal pressure. The pelvic plexus ganglia were pinned to the floor of the organ bath. All bath compartments were perfused separately with a modified Krebs solution at 37 °C, bubbled with 95% O_2, 5% CO_2, of the following composition (mM): 117 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.5 $NaHCO_3$ and 11 glucose.

Experiments to study reflexes between segments of distal colon and between colon and urinary bladder. Intraluminal pressure recording techniques were used to study *in vitro* the contractile responses of the colon and urinary bladder. In these experiments, two isolated segments of the colon or one segment of the colon and urinary bladder were attached by peripheral nerve trunks (colonic nerves, urethra/urinary bladder nerves; see Fig. 24 a, b) to the pelvic plexus ganglia. In the experiments where colon-colonic reflexes were studied, the *in vitro* preparation was placed in a three compartment organ bath. One colon segment was placed in one compartment, the other segment and pelvic plexus ganglia were placed separately in the other two compartments. In the experiments where colon-urinary bladder reflexes were studied, the colon segment and pelvic plexus ganglia were placed in one compartment of the organ bath while the urethra-urinary bladder was placed in the other. The nerve fibers which connect the peripheral organs and pelvic plexus ganglia were draped across a 1 mm thick wall separating the compartments and covered by moist strips of tissue paper to prevent dehydration. In experiments where the reflexes were studied by direct activation of the peripheral nerve fibers, stimulating electrodes were positioned on colonic nerves that connected one segment of the isolated distal colon with pelvic plexus ganglia while intraluminal colonic pressure was recorded from another distal colon segment to study

Figure 24. Diagrammatic sketch of *in vitro* preparations. A, Experiment to study reflexes between two segments of colon and B, reflexes between the colon and urinary bladder. (A), Two isolated segments of colon are connected to pelvic plexus ganglia by colonic-rectal nerves. (B), Colon and urinary bladder are connected to pelvic plexus ganglia via colonic-rectal nerves and urethra-urinary bladder nerves respectively.


A
ORAD
ABORAD
Proximal
Distal
Colonic N.
Colonic-Rectal N.
Hypogastric N.
Pelvic N.
Pelvic Plexus Ganglia
Urethra-Urinary Bladder N.
Pelvic Plexus Ganglia


B
ORAD
ABORAD
Proximal
Distal
Colonic N.
Colonic-Rectal N.
Hypogastric N.
Pelvic N.
Pelvic Plexus Ganglia
Urethra-Urinary Bladder N.
Pelvic Plexus Ganglia

the colon-colonic reflexes. To study colon-urinary bladder reflexes, the stimulating electrodes were positioned on colonic-rectal nerves and intraluminal pressure in the urinary bladder was recorded, or stimulating electrodes were positioned on urethra-urinary bladder nerves while the intraluminal colonic pressure was measured. Electrical stimulation was applied at frequencies ranging between 0.5 to 20 Hz (duration 0.5 ms at supramaximal intensities). Intraluminal pressure was recorded from the other colon segment or urinary bladder. Passive distension of the colon occurred in increments of 1 ml to maximum volume of 6 ml. Frequency dependent contractile response curves were constructed by expressing the contractile responses at different frequencies as a percentage of the maximal contractile responses to pelvic nerve stimulation.

Drugs were administered by separate superfusion to either the colon compartment, urethra-urinary bladder compartment or the ganglia compartment of the *in vitro* organ bath.

Data for mechanical activity was stored on a videocassette data recorder and the output (intraluminal pressure) was plotted on a pen-writing chart recorder.

Data were expressed as mean ± SEM. Student's *t* test was used for paired comparison. $p<0.05$ was considered as significant.

Results

Passive distension of one colonic segment or electrical stimulation of its colonic nerves (3-20 Hz at supramaximal voltage) evoked contractile responses of the other colonic segment (Figure 25A, B) and the urinary bladder (Figure 26). Electrical stimulation of urethra-urinary bladder nerves (5- 20 Hz) also evoked colon contractile responses (Figure 27). The contractile responses were frequency dependent (Fig. 28, 29, 30) and were abolished by: 1) sectioning the colonic nerves that connect the pelvic plexus ganglia with colon segment which had its intraluminal pressure recorded or the urethra-

Figure 25. Contractile responses of distal colon segment during passive distension of the proximal colon segment by injecting 3.5 ml of Krebs solution into the lumen (A) and electrical stimulation of colonic nerve fibers attached to proximal colon segment (B) at 10 Hz (a), 15 Hz (b) and 20 Hz. (C): contractile responses of distal colon segment evoked by electrical stimulation (20 Hz, 0.5 ms pulse duration, supramaximal voltage for 45 sec) of colonic nerves attached to proximal colon segment in the absence (left column) and presence (right column) of tetrodotoxin (TTX) (a), hexamethonium (C_6) (b), atropine (c) and after sectioning the colonic nerves (d).

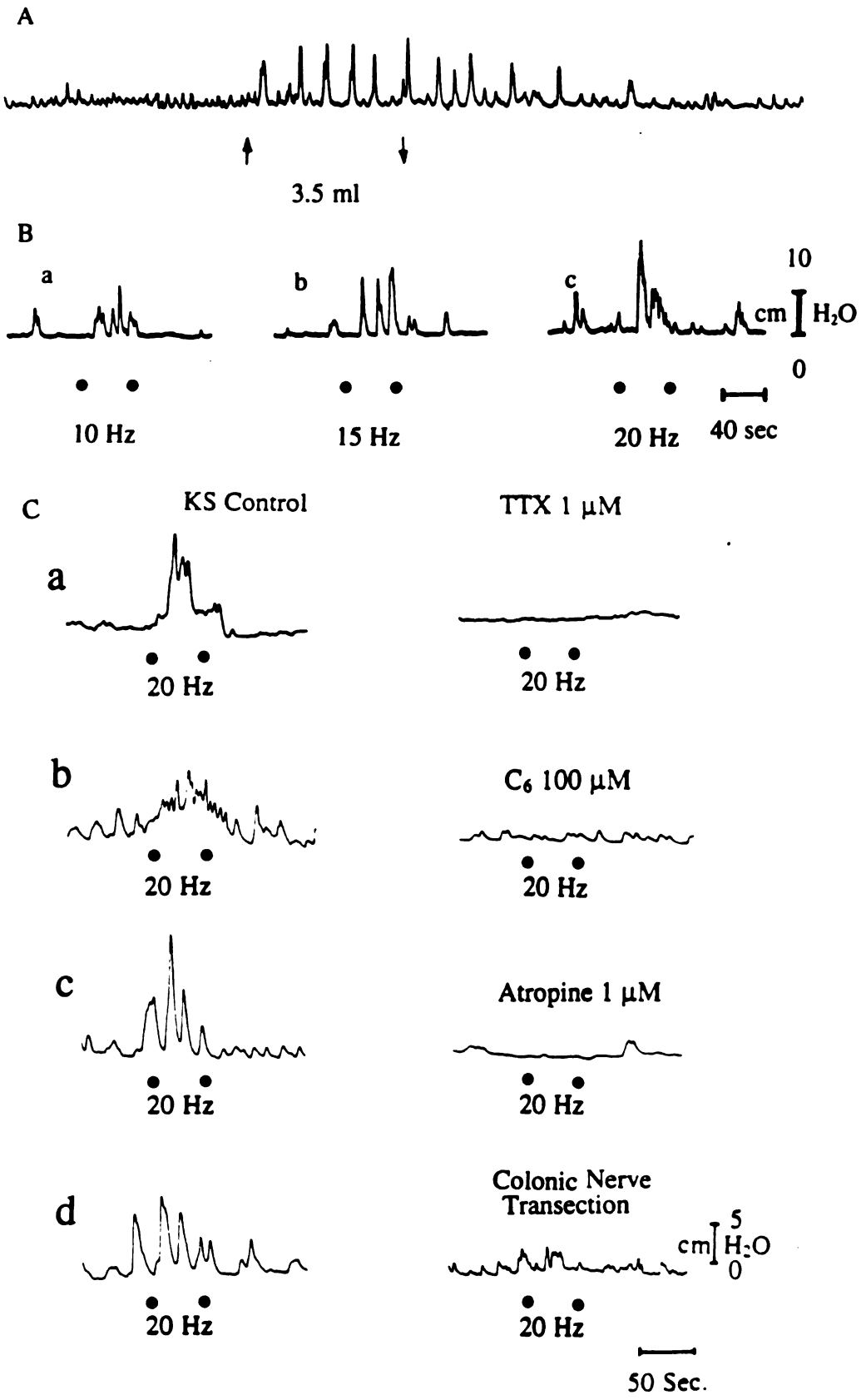

A
3.5 ml
B
a
b
c
10
cm
H2O
0
10 Hz
15 Hz
20 Hz
40 sec
C
KS Control
TTX 1 µM
a
20 Hz
20 Hz
b
C6 100 µM
20 Hz
20 Hz
c
Atropine 1 µM
20 Hz
20 Hz
d
Colonic Nerve Transection
5
cm
H2O
0
20 Hz
20 Hz
50 Sec.

Figure 26. Contractile responses of urinary bladder during passive distension of colon-rectum by injecting of a 5 ml Krebs solution into the lumen (a) and electrical stimulation of colonic-rectal nerves at frequency of 10 Hz (b, c, d). Left panel represents the urinary bladder contractile responses in a normal Krebs solution. Right panel represents the urinary bladder contractile responses during superfusion of the urinary bladder with a Krebs solution containing tetrodotoxin (TTX, 1 μM) (a, c) and atropine (1 μM) (b) and during superfusion of pelvic plexus ganglia with a Krebs solution containing hexamethonium (C_6, 100 μM) (d).

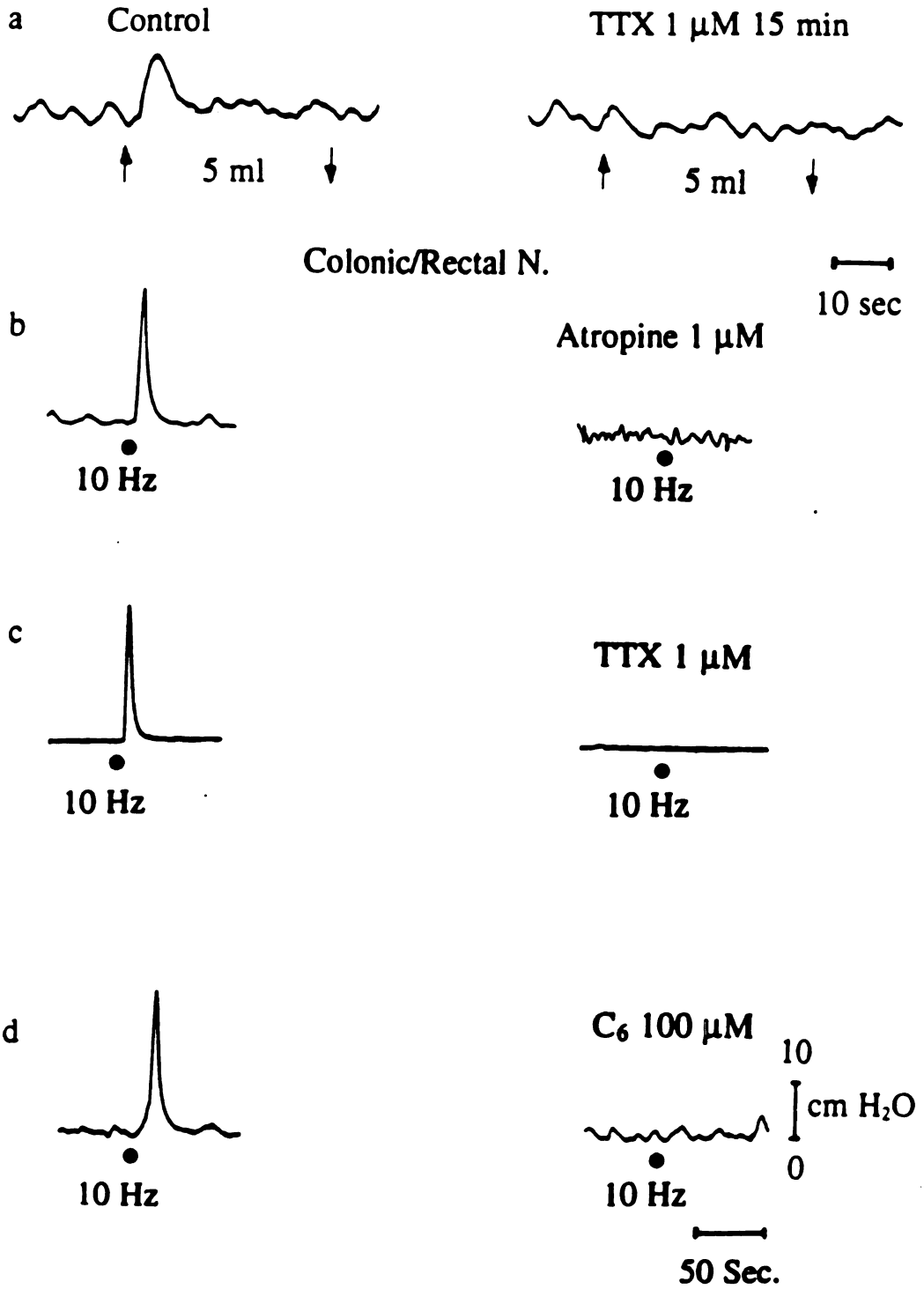

a
Control
5 ml
TTX 1 µM 15 min
5 ml
Colonic/Rectal N.
10 sec
b
10 Hz
Atropine 1 µM
10 Hz
c
10 Hz
TTX 1 µM
10 Hz
d
10 Hz
C6 100 µM
10
cm H2O
0
10 Hz
50 Sec.

Figure 27. A: Contractile responses of colon-rectum in normal Krebs solution evoked by electrical stimulation of urethra-urinary bladder nerves at frequency of 20 Hz.

B: Contractile responses of colon-rectum when electrical stimulation of urethra-urinary bladder nerves during superfusion of the colon-rectum with a Krebs solution containing tetrodotoxin (TTX, 1 μM) (top trace), atropine (1 μM) (bottom trace) and during superfusion of the pelvic plexus ganglia with a Krebs solution containing hexamethonium (C_6, 100 μM) (middle trace).

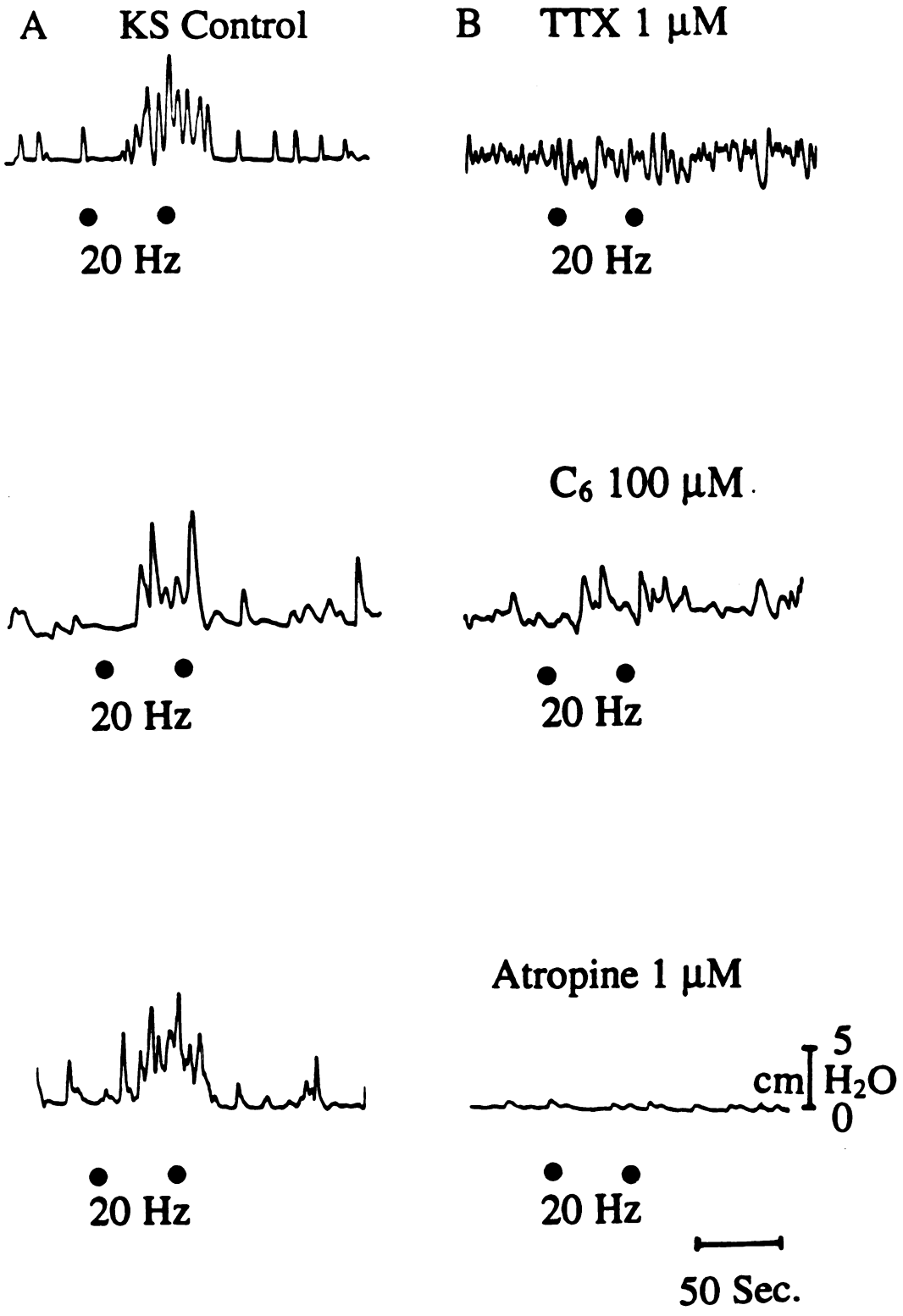

A KS Control
B TTX 1 µM
20 Hz
20 Hz
C6 100 µM
20 Hz
20 Hz
Atropine 1 µM
20 Hz
20 Hz
cm
5
H2O
0
50 Sec.

Figure 28. Effects of electrical stimulation of colonic nerve fibers originating from proximal colon segment on contractile responses recorded from distal colon segment (0.5 ms pulse duration, supramaximal voltage for 45 sec). A and B, frequency dependent contractile responses of distal colon segment during electrical stimulation of pelvic nerves and nerve fibers originating from proximal colon segment at indicated stimulating frequencies, respectively. Time period between two filled circles shows time of electrical stimulation. C: Frequency response curves of distal colon segment contractile responses. ■, represent pelvic nerve stimulation. ❑, represent the urethra-urinary bladder nerves stimulation. Ordinate, contractile responses expressed as a percentage of maximal pelvic nerve-evoked contractions. Abscissa, stimulation frequency. Values are mean ± S. E. Mean, n=4.

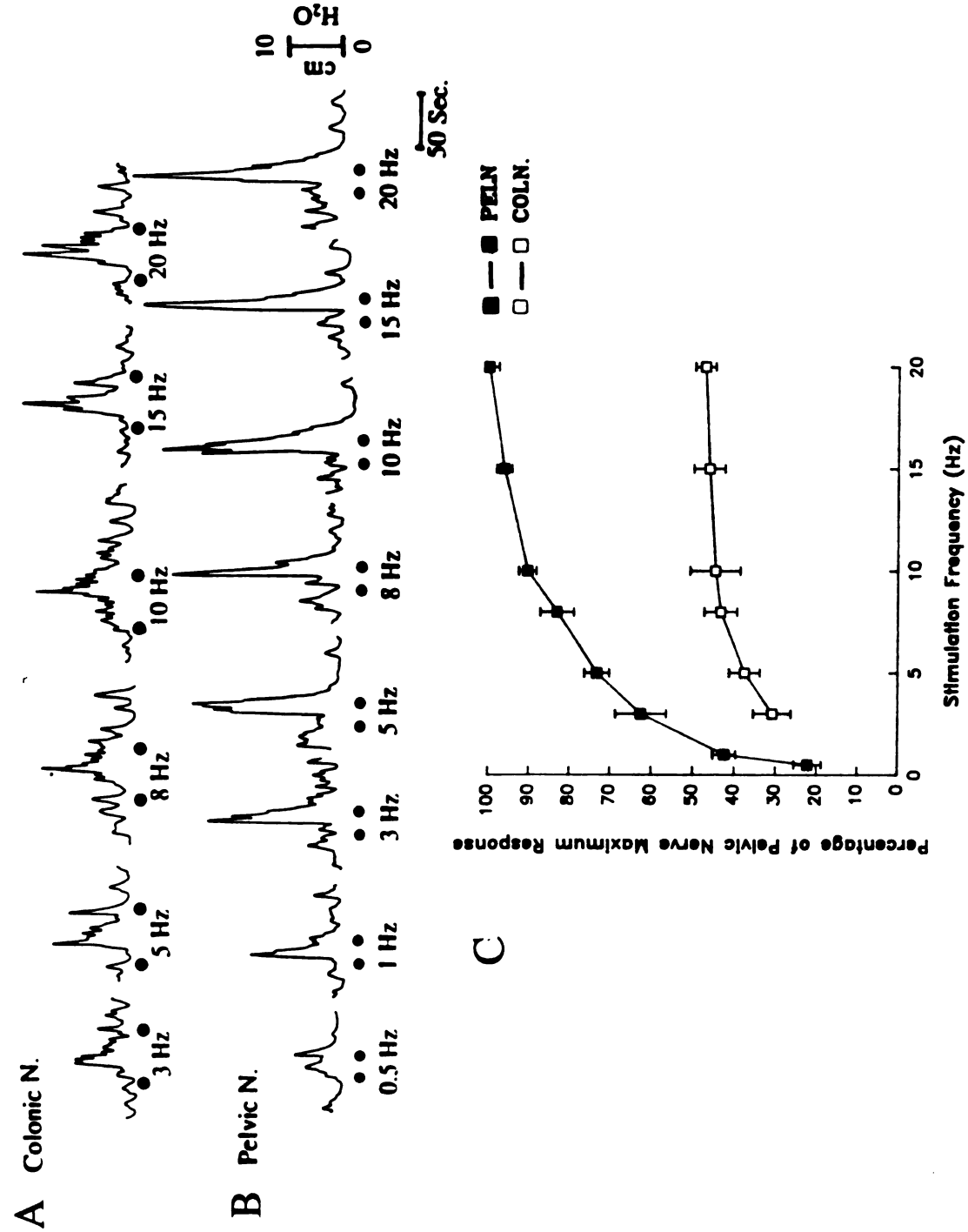
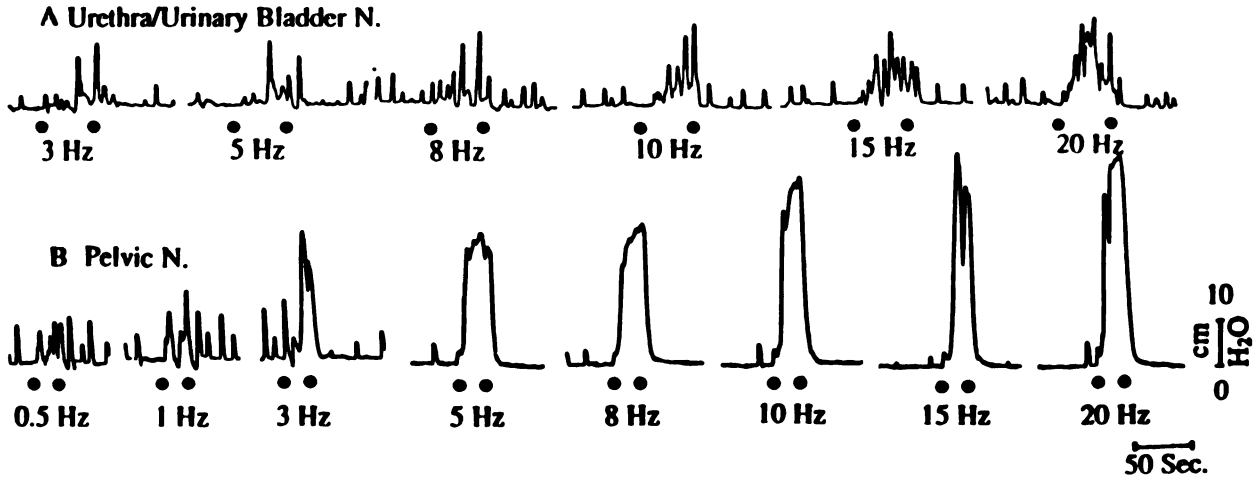

A Colonic N.
3 Hz
5 Hz
8 Hz
10 Hz
15 Hz
20 Hz
B Pelvic N.
0.5 Hz
1 Hz
3 Hz
5 Hz
8 Hz
10 Hz
15 Hz
20 Hz
10
0
cm H2O
50 Sec.
C
PELN
COLN.
Percentage of Pelvic Nerve Maximum Response
Stimulation Frequency (Hz)

Figure 29. Effects of electrical stimulation of urethra-urinary bladder nerves on contractile responses recorded from distal colon segment (0.5 ms pulse duration, supramaximal voltage for 30 sec). Time period between two filled circles shows time of electrical stimulation. A and B, frequency dependent contractile responses of colon-rectum during electrical stimulation of urethra-urinary bladder nerves and pelvic nerves at indicated stimulating frequencies, respectively. (B). C: Frequency response curves of distal colon-rectum contractile responses. ●, represents pelvic nerve stimulation. ○, represents urethra-urinary bladder nerve stimulation. Ordinate, contractile responses expressed as a percentage of maximal pelvic nerve-evoked contractions. Abscissa, stimulation frequency. Values are mean ± S. E. Mean, n=5.


A Urethra/Urinary Bladder N.
3 Hz
5 Hz
8 Hz
10 Hz
15 Hz
20 Hz
B Pelvic N.
0.5 Hz
1 Hz
3 Hz
5 Hz
8 Hz
10 Hz
15 Hz
20 Hz
10
cm H2O
0
50 Sec.

C

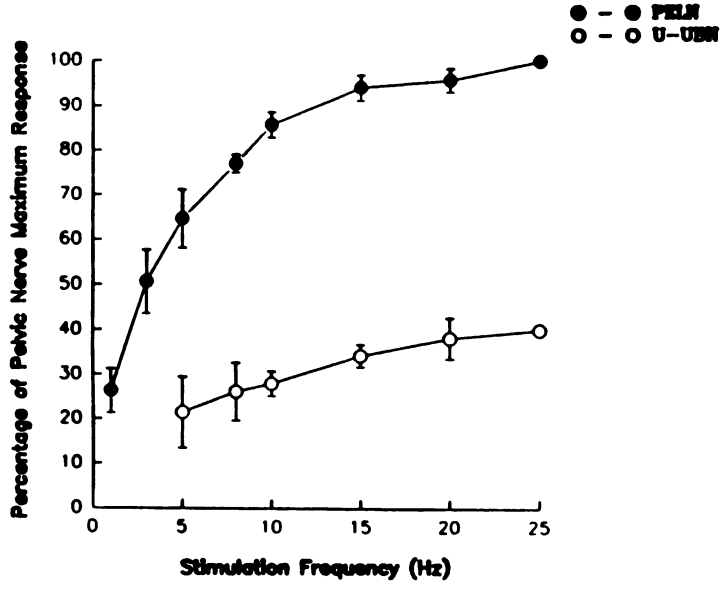
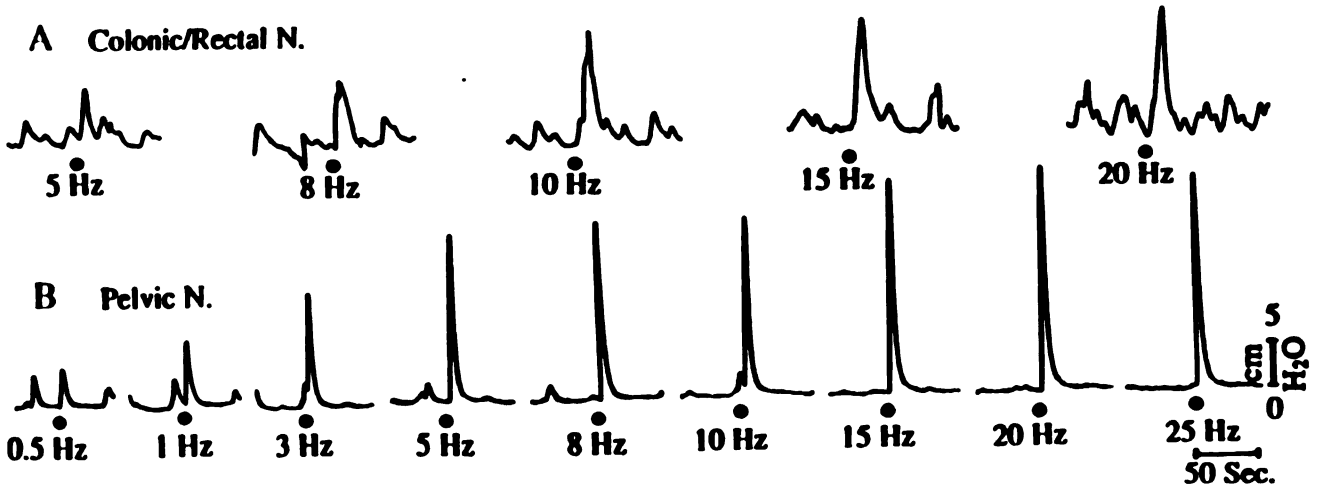
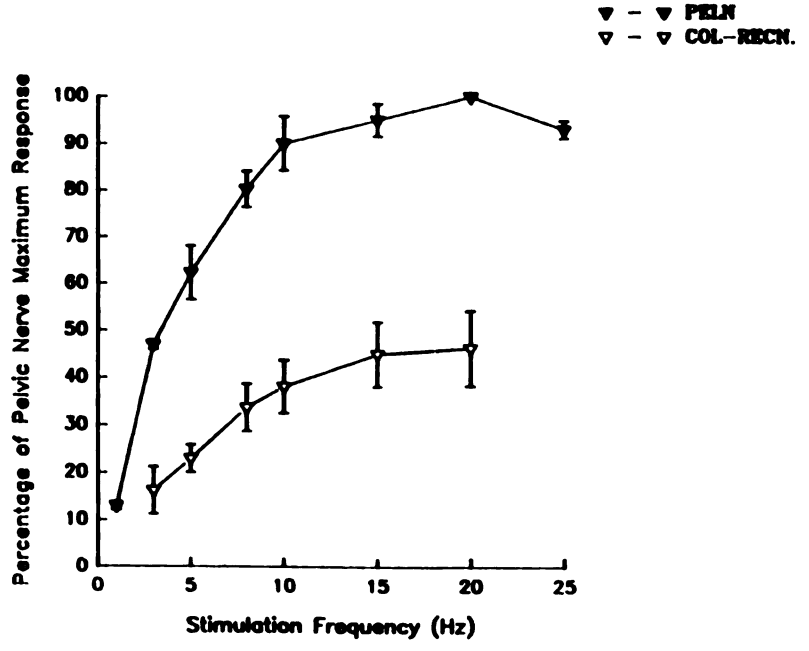

● – ● PELN
○ – ○ U–UBN
Percentage of Pelvic Nerve Maximum Response
0
10
20
30
40
50
60
70
80
90
100
0
5
10
15
20
25
Stimulation Frequency (Hz)

Figure 30. Effects of electrical stimulation of colonic-rectal nerves on contractile responses recorded from urinary bladder (0.5 ms pulse duration, supramaximal voltage for 10 sec). A and B, frequency dependent contractile responses of urinary bladder during electrical stimulation of colonic-rectal nerves and pelvic nerves at indicated stimulating frequencies, respectively. C: Frequency response curve of urinary bladder contractile responses. ▼, represents pelvic nerve stimulation. ∇, represents colonic-rectal nerve stimulation. Ordinate, contractile responses expressed as a percentage of maximal pelvic nerve-evoked contractions. Abscissa, stimulation frequency. Values are mean ± S. E. Mean, n=5.


A Colonic/Rectal N.
5 Hz
8 Hz
10 Hz
15 Hz
20 Hz
B Pelvic N.
0.5 Hz
1 Hz
3 Hz
5 Hz
8 Hz
10 Hz
15 Hz
20 Hz
25 Hz
50 Sec.
5
cm H2O
0

C


▼ - ▼ PELN
▽ - ▽ COL-RECN.
Percentage of Pelvic Nerve Maximum Response
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
Stimulation Frequency (Hz)

urinary bladder nerves that connected the pelvic plexus ganglia with urethra-urinary bladder which had its intraluminal pressure recorded, 2) superfusion of pelvic plexus ganglia with Krebs solution containing hexamethonium (10-100 μM), 3) superfusion of colon or urinary bladder with Krebs solution containing tetrodotoxin (1 μM) and 4) atropine (1 μM) (Fig. 25C, 26, 27) These data indicated that the contractions were neurogenic, mediated by fibers in CN and UUBN and involved nicotinic acetylcholine receptors and muscarinic receptors.

Effects of Acute Capsaicin Treatment. Effects of capsaicin on colon contraction evoked by electrical stimulation of urethra-urinary bladder nerve orcolonic nerves which attached to one of the two separated segments of the distal colon were studied. Capsaicin was used to determine if axon collaterals from primary afferents in pelvic and hypogastric nerve, which are components of the colonic and urethra-urinary bladder nerves, were involved in peripheral nerve stimulation-evoked colon contraction. The protocol used for acute "desensitization" of primary afferent fibers with cell bodies in the dorsal root ganglia involved treatment with 10 μM capsaicin for 45 min, followed by a 60 min washout period. Acute capsaicin "desensitization" was used to abolish the responses mediated by primary afferents but not by SP and CGRP-containing intramural neurons (Myers and Undem, 1991). Superfusion of pelvic plexus ganglia with a Krebs solution containing capsaicin (10 μM, 45-65 min, followed by 60-120 min wash) had no effect on electrical stimulation (8-15 Hz, 0.5 ms duration, supramaximal voltage) of distal colonic nerve-evoked contraction of the proximal segment of distal colon (n=2) and on colon contraction evoked by electrical stimulation of urethra-urinary bladder nerves (n=3) (Fig. 31). The data suggest that capsaicin-sensitive primary afferent fibers with cell bodies located in dorsal root ganglia, were not involved in these electrically-induced contractions.

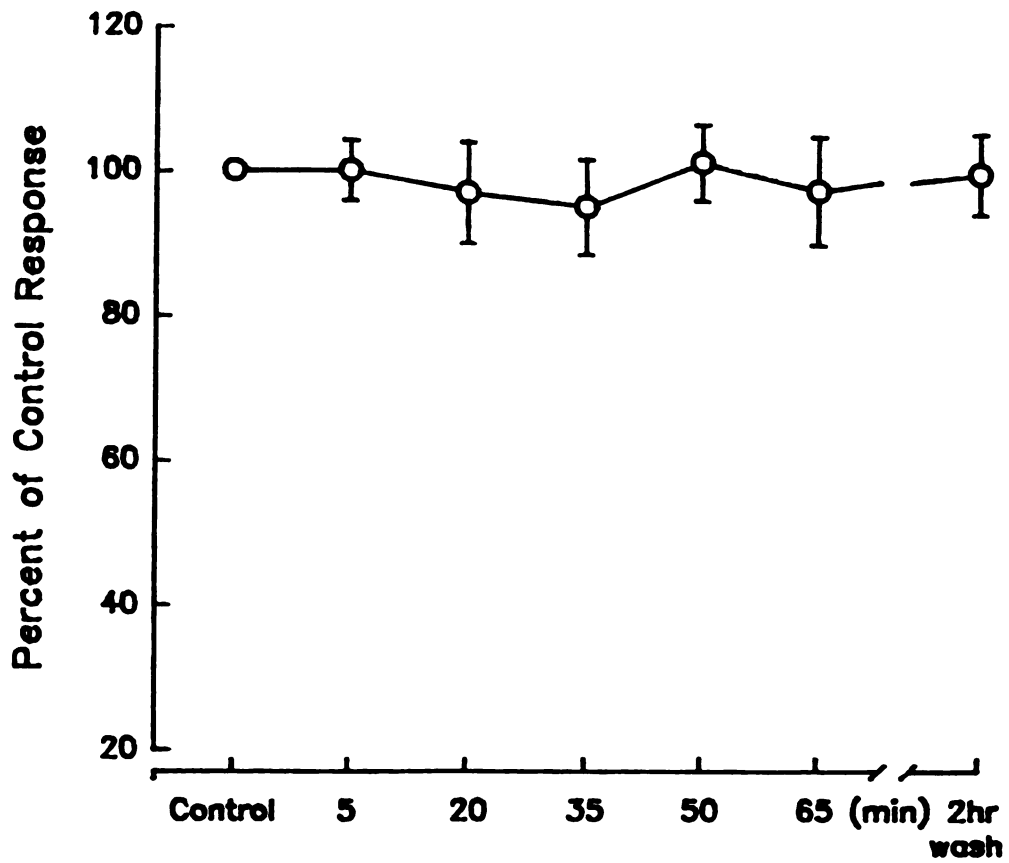


Figure 31. Effect of capsaicin (10 μM) on urethra-urinary bladder nerves (15 Hz, 35 sec.) evoked contractile responses of guinea-pig distal colon segment when superfused on pelvic plexus ganglia only, *in vitro*. Ordinate, percent of control contractile responses of distal colon. Abscissa, time period of capsaicin application.

Discussion

The present study shows that in preparations where peripheral organs were connected to each other via only the pelvic plexus ganglia and peripheral nerve trunks, electrical stimulation of nerve fibers connecting one segment of distal colon with pelvic plexus ganglia or distension of that colon segment evoked contractile responses of the other colon segment. Also, electrical stimulation of colonic nerves or distention of a colonic segment evokes contractile responses of urinary bladder. Furthermore, electrical stimulation of urethra-urinary bladder nerve evokes distal colon contraction. The reflex responses are blocked by peripheral nerve trunk transection, superfusion of hexamethonium on pelvic plexus ganglia and tetrodotoxin and atropine on the peripheral organ. This indicates that the excitatory reflexes between colon segments and between colon and urethra-urinary bladder are neurogenic, mediated through peripheral nerve fibers and pelvic plexus ganglia by cholinergic nicotinic and muscarinic receptors. An inhibitory colon-colonic reflex mediated by neurons in inferior mesenteric ganglia has previously been shown (Kreulen and Szurszewski, 1979b). It is known that the majority of neurons in pelvic plexus ganglia are cholinergic (Yokota and Burnstock, 1983) while the majority of neurons in inferior mesenteric ganglia are adrenergic (Szurszewski and Weems, 1976). Generally, afferent input to neurons in inferior mesenteric ganglia will increase sympathetic discharge to the colon which has been shown to decrease colonic contraction while afferent input to neurons in pelvic plexus ganglia will increase cholinergic discharge to the colon which has been shown to increase colonic contraction. This provides an explanation of the fact that neurons in pelvic plexus ganglia mediate excitatory reflexes and neurons in inferior mesenteric ganglia mediate inhibitory reflexes. The data also demonstrate that peripheral reflexes can be realized not only through prevertebral sympathetic ganglia but also through parasympathetic ganglia. In normal defecation and continence process or in patients with spinal cord transection, the

existence of this reflex pathway may be physiologically or pathologically significant. Under physiological condition, two general reflex pathways have been described. One of the reflex pathways is mediated through central nervous system at spinal cord level. The efferent limb of this reflex pathway involves both sympathetic, parasympathetic and somatic nervous system. This reflex pathway involves sending information to the brain. Another reflex pathway is locally defined. The reflex center is located on the wall of the intestine (mucosal and submucosal ganglia). This reflex is also called peristaltic reflex (Costa and Furness, 1976). The autonomic ganglia mediated reflex may be the third reflex pathway that mediates normal defecation and continence. Excitatory reflexes mediated by pelvic plexus ganglia may contribute to defecation while the inhibitory reflex mediated by inferior mesenteric ganglia may contribute to continence. Under pathological conditions, e.g. in spinal cord transacted patients, the reflex through central nervous system is abolished, defecation and continence are mainly dependent upon local peristaltic reflex and autonomic ganglia mediated reflex. Pelvic plexus ganglia mediated excitatory reflex may play a role in helping the defecation in these patients. Also from the inter organ reflex experiments, it has been shown that pelvic plexus ganglia mediate excitatory reflex between distal colon and urinary bladder. Under physiological conditions, the defecation reflex will initiate the micturition reflex and micturition reflex will also initiate the defecation reflex. The level of this type of reflex has been shown to be at the spinal cord (de Groat, Nadelhaft, Milne, Booth, Morgan and Thor, 1981). Based on the results of the present studies, we speculate that pelvic plexus ganglia may also involve in simultaneous micturition and defecation reflexes.

Although the general function of inferior mesenteric ganglia and pelvic plexus ganglia is to mediate inhibitory and excitatory reflexes, respectively, it is possible that opposite function of these ganglia also be obtained under certain conditions. It has been shown that when the colon smooth muscle tone is low, stimulation of postganglionic sympathetic fibers induces contraction of the circular layer of colon smooth muscle. This

response is mediated by co-release of noradrenaline and ATP or a related purine nucleotide from post ganglionic sympathetic nerve fibers part of which originate from inferior mesenteric ganglia, acting on postjunctional α_1-adrenoreceptors and P_{2x}-purinoceptors (Venkova and Krier, 1993, 1994). When colon smooth muscle tone is high, stimulation of postganglionic parasympathetic nerve fibers induced relaxation of colon smooth muscle. This relaxation is mediated by nonadrenergic, noncholinergic (NANC) enteric neurons (Zeitlin, Johnson, Fasth, Hulten and Eaglesom, 1978). Cholinergic postganglionic parasympathetic nerve fibers originated from pelvic plexus ganglia is one source of activation of the NANC inhibitory neurons. Although inferior mesenteric ganglia and pelvic plexus ganglia have been demonstrated to mediate excitatory and inhibitory reflexes, respectively when colon or intestine are under normal muscle tone , I speculate that these reflexes can also be altered when the smooth muscle condition is different.

Acute capsaicin treatment depletes tachykinins (including substance P) and calcitonin gene-related peptides from primary afferent terminals (Myers and Undem, 1991) in the hypogastric and pelvic nerves which may give axon collaterals to pelvic plexus ganglia. The data show that acute capsaicin treatment of pelvic plexus ganglia has no effect on nerve stimulation-evoked excitatory reflex responses, suggesting that axon collaterals from primary afferent fibers are not involved in pelvic plexus ganglia mediated peripheral reflexes. The present data also in accordance with data presented in Chapter 3, which show no slow synaptic potentials in pelvic plexus ganglion neurons during passive distension of the distal colon segment. If capsaicin-sensitive primary afferent fibers were involved in the afferent arc of reflexes, tachykinins released from the capsaicin sensitive primary afferent terminals during distension should have caused slow membrane depolarization, as shown in neurons of inferior mesenteric ganglia (Kreulen and Peters, 1986). Application of tachykinins including NKB and substance P have also been shown

to depolarize inferior mesenteric ganglion neurons and bronchial parasympathetic ganglion neurons (Dun and Minota, 1981; Myers and Undem, 1993).

Since the central nervous system is also a source of excitatory synaptic input to pelvic plexus ganglion neurons through the pelvic and hypogastric nerves, it is possible that pelvic plexus ganglia is capable of modifying the level of mechanoreceptor input it receives from the distal colon by integrating the peripheral input with input from the central nervous system.

In summary, the present studies show that excitatory reflexes between colon segments and between the colon and the urinary bladder can be mediated by neurons in pelvic plexus ganglia. The peripheral reflexes are mediated by nicotinic and muscarinic cholinergic neurotransmission. Axon collaterals from capsaicin-sensitive primary afferent fibers are not involved in these pathways.

CHAPTER 5
THE INTERACTION OF INFLAMMATORY MEDIATORS WITH PELVIC PARASYMPATHETIC SYSTEM.

Introduction

Inflammation of the gastrointestinal system.

Tissue injury or infection is accompanied by a complex series of homeostatic reactions, involving the immune, circulatory and nervous system, that is termed inflammation. In cutaneous tissues, the processes of inflammation lead to four well pronounced clinical signs: heat, redness, edema and pain (Gauldie, 1991). These responses are caused by vasodilation, plasma extravasation and sensitization and activation of nociceptive C-fiber nerve endings (Sharkey, 1992). It is also well known that visceral inflammation is associated with persistent pain and dramatic gastrointestinal motility changes as a consequence of an exaggerated reflex activity. Enhanced sensitivity of the viscera in inflammation has been well documented. Mechanical stimuli such as light pressure or distension do not elicit any sensation or reflex when applied to the healthy mucosa of gastrointestinal or urinary tract. However, when these tissues become chronically inflamed, their sensitivity markedly increases and formerly unnoticeable stimuli can elicit considerable pain, discomfort and reflex motility changes (Jänig and Koltzenburg, 1990).

The development of prolonged hyperalgesia and hyperreflexia are prominent findings of the inflammatory process in visceral organs resulting in the presence of discomfort, exaggerated perception of pain, abdominal cramps, dysuria, urgency, increased frequency of micturition and frequent bouts of severe diarrhea. Thus, visceral tissues can become the source of pain and on-going tenderness during inflammation,

although they are largely insensitive to a wide variety of stimuli under normal non-inflamed conditions (Mclellan and Goodell, 1943; Wolf, 1965).

Spinal visceral afferents supplying the colon and the urinary bladder are involved in neural reflex regulation of continence and evacuation and in perception of different sensations occurring under physiological and pathological conditions (de Groat, Nadelhaft, Milne, Booth, Morgan and Thor, 1981; Haupt, Jänig and Kohler, 1983; Häbler, Jänig and Koltzenburg, 1988). How visceral primary afferents encode peripheral events and how central neurons decode these messages leading to appropriate reflex patterns and sensations have been studied both under physiological and pathological conditions. Distension and contraction of colon and urinary bladder elicit autonomic reflexes and sensations in humans and animals. The sensations are generally diffuse and consist of feelings of fullness and urgency to void at low intraluminal pressures and are gradually supplanted by discomfort and pain when pressures exceed physiological values. Pathological changes during inflammation, may elicit spontaneous pain that is different in character from visceral pain elicited under physiological conditions (Jänig, Koltzenburg, 1989; Jänig and Morrison, 1986).

For neurophysiological investigations, people have studied the visceral sensory mechanism under pathological conditions. To stimulate an acute inflammatory process, algesic chemicals, such as bradykinin or capsaicin have been applied topically onto afferent endings or have been injected intra-arterially into the vascular supply of the organ (Bahns, Ernsberger, Jänig and Nelke, 1986; Haupt, Jänig and Kohler, 1983). These substances activate the majority of mechanosensitive afferents that also respond to distension of the organ. To investigate the neural substrates of visceral inflammatory hyperalgesia and hyperreflexia, intraluminal injection of chemical irritants such as mustard or turpentine oil was performed (Häbler, Jänig and Koltzenburg, 1988, 1989). Mechanosensitive vesical afferents are vigorously excited by either irritant. Both inflammatory algesic chemicals and inflammatory chemical irritants produced

hyperalgesia and hyperreflexia that resembled the inflammatory disease of visceral organs.

Inflammatory bowel diseases (IBD) (Crohn's disease and ulcerative colitis) are a group of chronic illnesses characterized by periods of active disease followed by remission and relapse with symptoms of bouts of diarrhea, cramping of the abdomen and fever. The IBD responses include infiltration of plasma cells, mast cells, macrophages and lymphocytes into the mucosa and, although it is a chronic disease, a prominent acute phase involving local granulocyte immigration (Kirsner and Shorter, 1988) also occurs. The original observation that ganglion cells were increased in the enteric nervous system in IBD, established a neural involvement in the diseases (Sharkey, 1992). In addition to the neural hyperplasia found in some tissues, necrosis, degeneration of ganglion cells and axons have also been observed (Koch, Sonnenberg and Carney, 1991). These pathological abnormalities appear to be dependent on the disease (Crohn's vs. ulcerative colitis), the region of the intestinal wall (mucosa vs. muscle) and whether or not the tissue was from a site of active disease (Koch, Sonnenberg and Carney, 1991). Intensive studies involving measurements of neurotransmitter content in the gut wall of IBD patients have prompted a systemic study of the impact of inflammation on neurotransmitter content and release in the myenteric plexus in animal models. The studies have shown that inflammation decreases acetylcholine release from myenteric terminals in the inflammatory intestine of rats (Collins, Hurst, Main, Stanley, Khan, Blennerhassett and Swain, 1992). Furthermore, the inhibition of release of acetylcholine is mediated by the inflammatory mediator, interleukin-1β. Increased production of interleukin-1 occurs in patients with active IBD (Ligumsky, Simon, Karmeli and Rachemilewitz, 1990) and interleukin-1 is expressed early during the course of experimental colitis in rabbits (Cominelli, Nast, Clark, Schindler, Lierena, Eysselein, Thompson and Dinarello, 1990) where it may play a protective role (Cominelli, Nast,

Lierena, Dinarello and Zipser, 1990). These findings prompted an evaluation of the ability of IL-1β to influence neurotransmitter content and release in rat myenteric plexus.

The studies in the previous chapters have shown that neurons in pelvic plexus ganglia are able to mediate peripheral visceral organ reflexes. It is important to study this reflex not only under physiological conditions but also under pathological conditions such as inflammation. From the broad spectrum of inflammatory mediators, bradykinin and interleukin-1β were selected in the present study because of: 1) their prominent effects as inflammatory mediators; 2) the specific effects of bradykinin on mechanosensitive receptors located in the visceral organs; 3) the important role that interleukin-1β plays as a communicator between immune system and nervous system during inflammation.

Actions of bradykinin on visceral afferent fibers.

Bradykinin (BK) is a nonapeptide which is released from ischemic and inflamed tissues (Keele and Armstrong, 1964). Normally, blood and tissue levels of BK are very low. Upon injury, a cascade of biochemical reactions is initiated, which results in the proteolytic generation of BK and a further kinin (kallidin; lys-BK) from high molecular weight precursors, the kininogens, found in blood and tissue (Miller, 1987). The actions of BK are mediated through at least two different types of receptor designated as BK_1 and BK_2 (Regoli, Rhaleb, Dion and Drapeau, 1990). Most biological actions of BK seem to be mediated by BK_2-receptors. BK can elicit either contraction or relaxation of vascular smooth muscle depending on the animal species and site of action. BK also produces contraction of smooth muscles of the respiratory and gastrointestinal tract and the uterus. In several species exogenously applied kinins have been shown to produce the cardinal symptoms of inflammation (Wihelm, 1973). BK is also called a vasoneuroactive agent because it causes vasodilation and increases capillary permeability leading to edema (Guzman et al, 1962; Handwerker, 1980; Lim et al, 1962). BK was

found to release prostaglandins and leukotrienes through activation of phospholipase A_2 (Griesbacher and Lembeck, 1987). Exciting nociceptors and stimulating nociceptive afferent nerves, BK mediates pain and hyperalgesia (Juan and Lembeck, 1974; Steranka, Manning, de Haas, Ferkany, Borosky, Connor, Vavrek, Stewart, and Snyder, 1988).

Bradykinin is known to be one of the most potent algesic substances formed in the body and is a potent inflammatory mediator in inducing acute inflammation. The hyperalgesic and pro-inflammatory activity of BK is either due to a direct action of BK on a subpopulation of nociceptive primary afferent nerve terminals through the activation of a receptor-coupled enzyme phospholipase C (Haupt et al., 1983; Miller, 1987; Mizumura et al., 1990) or indirectly through BK-induced release of other inflammatory mediators including cytokines (Ferreira, Lorenzetti, Cunha and Poole, 1993). In turn, cytokines further induce the release of hyperalgesic mediators including BK. Activation of phospholipase C results in the production of two intracellular messengers, inositol 1,4,5-triphosphate (IP_3) which stimulates intracellular calcium release and diacylglycerol (DAG) which activates protein kinase C. Protein kinase C phosphorylates membrane ion channel proteins to induce changes in ion permeability (Kaczmarek, 1987). The action of BK on visceral afferent fibers may also be due to the release of prostaglandins (Kumazawa and Mizumura, 1980). For example, prostaglandin E_2 sensitizes primary afferent terminals to BK (Mense, 1977; Handwerker, 1976; Chahl and Iggo, 1977; Ohno et al., 1984; Mizumura et al, 1987). The pain and inflammation induced by bradykinin are blocked by aspirin or other acidic non-steroidal anti-inflammatory drugs, which are known to inhibit cyclooxygenase and to suppress the formation of prostaglandins and thromboxanes. These data indicate that bradykinin produces pain and inflammation in part through the formation of prostaglandins (Katori, Hori, Uchida, Tanaka and Harada, 1986).

Although BK is used as a nociceptive stimulus (Guilbaud et al., 1976), it does not stimulate only nociceptors. BK also excites afferents that most likely are not involved in

nociception. For example, BK stimulates pulmonary and cardiac vagal C-fibers (Kaufman et al., 1980 a, b), vagal afferents of the duodenum (Cottrell and Iggo, 1984), and cutaneous low-threshold mechanoreceptors (Beck and Handwerker, 1974). Regarding the BK action on sensory afferents, there are two possibilities: 1) it can act directly on chemosensitive nerve endings (Baker et al., 1980; Cervero and Sharkey, 1988; Kaufman et al., 1980b; Longhurst et al., 1984) or 2) it can stimulate pure mechanoreceptors secondary to muscle contraction (Floyd et al., 1977; Kaufman et al., 1980a). However, it has been shown that all colonic distension-sensitive mechanoreceptors can be stimulated by a direct action of BK on nerve endings (Haupt et al., 1983). BK-induced depolarization of primary afferent nerve terminals has been shown in neonatal rats. The depolarization is due to an increase in sodium permeability of the membrane, which is mediated via the activation of PKC and DAG (Dunn and Rang, 1990).

Application of bradykinin to rat dorsal root ganglion neurons (Burgess, Mullaney, McNeill, Dunn and Rang, 1989), guinea-pig nodose ganglia (Undem and Weinreich, 1993) and hamster submandibular ganglion neurons (Suzuki, 1992) caused a depolarization associated with an inward current and an increase in membrane conductance that was probably due to the opening of the sodium channels. Like the mechanism involved in depolarization of the primary afferent terminals, opening of the sodium channels is mediated by PKC and DAG (Burgess, Mullaney, McNeill, Dunn and Rang, 1989). No hyperpolarization or outward current was detected. In dorsal root ganglion neurons, bradykinin also increases calcium influx and intracellular calcium concentration (Burgess, Mullaney, McNeill, Dunn and Rang, 1989) and cyclic-GMP concentration (McGehee, Goy and Oxford, 1992). The activation of guanylate cyclase by bradykinin is mediated by the influx of calcium (Burgess, Mullaney, McNeill, Coote, Minhas and Wood, 1989). The influx of calcium is nifedipine sensitive indicating that L-type voltage activated calcium channels are involved. Furthermore, bradykinin

stimulates phosphoinositide hydrolysis and mobilization of arachidonic acid in dorsal root ganglion neurons (Gammon, Allen and Morell, 1989). Elevated cyclic GMP inhibited bradykinin-induced formation of inositol phosphates, pointing a possible role of cyclic GMP in the regulation of polyphosphoinositide turnover in dorsal root ganglion neurons (Burgess, Mullaney, McNeill, Coote, Minhas and Wood, 1989).

The action of bradykinin on smooth muscle was characterized in guinea-pig taenia ceci. The bradykinin response was characterized by an initial hyperpolarization and suppression of spike activity followed by a sustained depolarization and an increased spike activity. Changes in membrane activity were accompanied by inhibition of phasic contractions followed by an increase in muscle tone and the development of phasic contractions. Membrane conductance was decreased during the sustained depolarization. The multiple actions of bradykinin are mediated via BK_2 receptors and involve: 1) calcium mobilization associated with activation of potassium channels; 2) calcium release from intracellular stores and 3) receptor-activated sodium channels (Hertog, Nelemans and den Akker, 1988).

The development of tachyphylaxis or desensitization to BK is also a well-recognized phenomenon. The pain evoked by application of BK on blister base skin diminishes in intensity with repeated applications (Elliott et al., 1960). Similar tachyphylaxis has also been observed in cardiac sympathetic afferents of the cat (Baker, Coleridge, Coleridge and Nerdrumet, 1980). It was suggested (McGehee, Goy and Oxford, 1992) that a nitric oxide-cyclic GMP pathway is involved in the mechanism of desensitization to BK at the receptor or G-protein level because: 1) desensitization to BK was enhanced by nitroprusside (NP) and reduced by nitric oxide synthase (NOS) inhibitors; 2) the effects of NOS inhibitors were overcome by 8 Br-cyclic GMP or L-arginine; and 3) 8 Br-cyclic GMP-evoked modification of desensitization to BK required receptor occupancy.

Bradykinin will be used in the experiments involved in this section of the dissertation, since it is known to activate mechanosensitive primary afferent fibers and to exert pro-inflammatory effects in the gastrointestinal system. The effects of BK on mechanoreceptor mediated responses in the *in vitro* distal colon-pelvic plexus ganglia preparation will be studied to elucidate if BK affects the afferent arc of the peripheral reflexes, which are mediated by neurons in pelvic plexus ganglia. The data show that application of bradykinin to the distal colon initiates and/or potentiates the mechanosensitive synaptic inputs to neurons in the pelvic plexus ganglia. The action of BK is due to either a direct effect on afferent terminals or to secondary activation of mechanoreceptors located in the distal colon by bradykinin-induced colon contractions .

Inflammatory mediator interleukin-1ß

At a conference held in the summer of 1984 in London, Guido Majno pointed out some 100 molecules, including about 50 lymphokines, which could claim to mediate some aspect of the inflammatory process. By the year 2000, this number would be considerably greater if the current rate of discovery were maintained (Majno, 1985). Over the last few years, evidence for an ordered control of the inflammatory process has begun to emerge in the sense that inflammation is one of the integral parts of the body defense mechanisms wherein the "50" or so lymphokines exercise considerable influence. Majno used the term lymphokine in the way others use cytokine, to define polypeptide mediators, released from a variety of cell types (not just lymphocytes), which activate, modulate and control various aspects of body defense and repair. Biologists had long suspected the existence of an overall control process for activating and co-ordinating the body defense which now appears to involve the synthesis and release of a number of cytokines, particularly interleukin 1 (IL-1) (Billingham, 1987).

Historically, IL-1 was "born" in 1972 during the course of *in vitro* studies by Gery and Waksman (Gery and Waksman, 1972). It was described as a necessary factor

for thymocyte and lymphocyte proliferation, and was given the title of "lymphocyte activation factor". Around the same time, other research groups were working on factors which activated other aspects of the inflammatory and defense mechanisms. Wood and coworkers were working on a factor necessary for B cell proliferation and antibody production, which they termed B cell activation factor (Wood and Gaul, 1974). Atkins, among others, was attempting to purify endogenous pyrogen (Bernheim, Block and Atkins, 1979), and Kampschmidt was working with leucocyte endogenous mediator (LEM) which activates the acute phase reaction, particularly the increased hepatic synthesis of acute phase plasma proteins (Kampschmidt, 1981). Other factors discovered in the mid to late-1970s like mononuclear cell factor (MCF) and catabolin (Dayer, Robinson and Krane, 1977; Dingle, Saklatvala, Hembry, Tyler, Fell and Jubb, 1979), possessed the ability to stimulate proteolytic enzymes and prostaglandins. The difficulties surrounding the attempts to physically separate endogenous pyrogen from LEM, lead to the suggestion that they might be the same molecule. Following the sharing of purified products between the various research groups, it rapidly became apparent that they were all studying essentially the same molecule. The various synonyms became included in the general title interleukin 1 in a specially convened workshop in 1979 (Aarden, Brunner and Cerottini, 1979), differentiating these properties from interleukin 2, which is a T cell product and is a growth factor for other lymphocytes.

It is now known that IL-1 is a 17.5 killodalton polypeptide synthesized and released by blood activated macrophages, endothelial cells, astrocytes and vascular smooth muscle cells during infection, injury, stress or antigenic challenge (Dinarello, 1985; 1988). It is a prominent member of a group of polypeptide mediators called cytokines. IL-1, initially synthesized as a 31 KD precursor that is subsequently processed to a 17.5 KD molecule, exists in two forms, interleukin-1α and interleukin-1ß. There are 26% amino acid homology between the two forms which are encoded by separate genes

on chromosome 2. Interleukin-1α remains cell associated while interleukin-1ß is secreted by blood monocytes/macrophages, T-lymphocytes, fibroblasts, endothelial cells, epithelial cells, adrenal chromaffin cells, astrocytes, skin keratinocytes and smooth muscle cells. Synthesis of interleukin-1ß mRNA is predominant over interleukin-1α in rodent and human cells (Dinarello, 1988). Two subtypes of interleukin-1 receptor have been cloned and are called type I and type II (Sims, March, Cosman, Widmer, MacDonald, McMahan, Grubin, Wignall, Jackson and Call, 1988). The type I receptor is a 80 KD glycoprotein and the type II receptor is a 68 KD glycoprotein. Both types have two ß-pleated sheets that are connected by disulfide bonds, which places them into the immunoglobulin superfamily. There is 28% homology in extracellular domains between the two receptor types. The type I receptor has 213 amino acid residues in its cytoplasmic domain while the type II receptor has only 29 amino acid residues in its cytoplasmic domain. These data strongly indicate that only the type I receptor is capable of transducing a signal and it can produce all of the biological effects attributed to IL-1 (Stylianou, O'Neill, Rawlinson, Edbrooke, Woo and Saklatvala, 1992; Sims, Gayle, Slack, Alderson, Bird, Giri, Colotta, Re, Mantovani and Shanebeck, 1993).

Following the binding of IL-1 to its receptor, two pathways for signal transduction have been proposed, (i): cyclic-AMP activation of protein kinase A; and (ii): phosphatidylcholine hydrolysis (Mizel, 1989). The inflammatory properties of IL-1 have been shown to induce the release of interleukin-1 itself, other cytokines, prostaglandin E_2, collagenase and phospholipase A_2 from immune cells, endothelial cells and smooth muscle cells and induce immune cell proliferation (Mizel, 1989). It also acts as a potent chemoattractant for leukocytes into the sites of active inflammation and involves tissue repair by increasing fibroblast proliferation and collagen synthesis (Eastgate, Symons and Duff, 1988).

A naturally occurring inhibitor of the biological activities of IL-1 has been purified and prepared by Synergen Inc. It is a 25 KD protein with 26% homology to IL-

1ß and 19% homology to IL-1α. It does not interact with IL-1α or IL-1ß, but binds to type I IL-1 receptor with an affinity approximately equal to IL-1α or IL-1ß. This protein is called IL-1 receptor antagonist (IL-1ra) (Hannum, Wilcox, Arend, Joslin and Dripps, 1990; Eisenberg, Evan, Arend, Verderber, Brewer, Hannum and Thompson, 1990). The current evidence indicates that the inhibitory action of IL-1ra results from competition with IL-1α or IL-1ß to the type I receptor and that the binding of IL-1ra to this receptor does not result in signal transduction (Dripps, Verderber, Ng, Thompson and Eisenberg, 1991; Granowitz, Clark, Mancilla and Dinarello, 1991). It has been shown that IL-1ra is released *in vivo* during inflammation and during the natural course of many diseases (Dinarello and Thompson, 1991). Administered experimentally, IL-1ra has been demonstrated to block IL-1 activity both *in vitro* and *in vivo*. In rabbits, pretreatment with IL-1ra has been shown to prevent death resulting from septic shock (Ohlsson, Bjork, Bergenfeldt, Hageman and Thompson, 1990), to prevent the development of immune complex-induced colitis (Cominell, Nast, Clark, Schindler, Lierena, Eysselein, Thompson and Dinarello, 1990) and to block cerebrospinal fluid inflammation induced by intracerebroventricular administration of IL-1 (Ramilo, Mustafa, Porter, Saez-Llorens, Mertsola, Olsen, Luby, Beutler and McCracken, 1990). Currently, pre-clinical and clinical studies are being carried out on the possible therapeutic uses of IL-1ra in the treatments of sepsis, cachexia, rheumatoid arthritis and chronic myelogenous leukemia.

The nervous and immune systems consist of complex networks of cells that monitor specific signals and respond in a specific manner to these signals. The nervous system, through the production of neuroregulators (neurotransmitters, neuromodulators and neuropeptides), can regulate specific immune system functions. The immune system produces a variety of chemical factors that exert specific immunoregulatory actions. These immunoregulators (immunomodulators and immunopeptides, which are substances with either stimulating or inhibiting activities on certain functions of the immune system)

also possess specific neuromodulatory activities. This establishes a bi-directional communication between the nervous and immune systems.

In the central nervous system, brain intrinsic and blood-derived macrophages (Jordan and Thomas, 1988), cerebrovascular endothelial cells (Billingham, 1987), microglia (Giulian, Allen, Baker and Tomozawa, 1986), astrocytes (Billingham, 1987) and even neurons (Breder, Dinarello and Saper, 1989) have the ability to synthesize and release IL-1ß in response to appropriate stimuli. IL-1β causes fever production (Nakashima, Hori, Mori, Kuriyama and Kizuno, 1989), suppresses food intake (McCarthy, Kluger and Vander, 1986; Mrosovsky, Molony, Conn and Kluger, 1989; Plata-Salamán, Oomura and Kai, 1988) and alters slow wave sleep (Dinarello, 1985). In hypothalamic brain slices, IL-1ß increases or decreases neuron firing of glucosensitive and thermosensitive neurons and depolarizes or hyperpolarizes supraoptic neurons (Nakashima, Hori, Mori, Kuriyama and Mizuno, 1989; Kuriyama, Hori, Mori and Nakashima, 1990; Li, Inenage, Kawano, Kannan and Yamashita, 1992). The effects of IL-1ß on thermosensitive neurons were blocked by sodium salicylate, an aspirin like drug and a cyclooxygenase inhibitor which is known to block prostaglandin E synthesis from arachidonic acid but were not blocked by naloxone (Hori et al., 1988). In rat hippocampal neurons, IL-1ß inhibits long term potentiation (Bellinger, Madamba and Siggins, 1993) and depresses a voltage dependent calcium current through protein kinase C signal transduction pathway (Palat-Salamán and Ffrench-Mullen, 1992; 1994). The action of IL-1β on primary afferents has been known to generally and critically involve in hyperalgesia induced by inflammatory processes (Ferreira, Lorenzetti, Cunha and Poole, 1993; Watkins, Wiertelak, Goehler, Smith, Martin and Maier, 1994).

In the gastrointestinal tract, IL-1ß inhibits basal and evoked release of norepinephrine and acetylcholine from myenteric nerve terminals (Collins, Hurst, Main, Stanley, Khan, Blennerhassett and Swain, 1992; Hurst and Collins, 1993; Main,

Blennerhassett and Collins, 1993). Its actions on the extrinsic autonomic neurons that innervate the gastrointestinal tract are yet not known.

The aim of the present study was to determine the effects of interleukin-1ß on the active and passive membrane properties and synaptic transmission of neurons in guinea-pig pelvic plexus ganglia. These neurons are the origin of postganglionic fibers that innervate vascular and visceral smooth muscle of colon, anal canal, urethra, urinary bladder and reproductive organs. They receive central synaptic inputs from fibers in pelvic and hypogastric nerves and peripheral synaptic inputs from fibers in urethra-urinary bladder and colonic-rectal nerves (Lin and Krier, 1993). Utilizing *in vitro* intracellular microelectrode techniques, we show that IL-1ß causes membrane depolarization, membrane hyperpolarization, blockade of orthodromic action potentials and depression of fast excitatory postsynaptic potentials (f-EPSPs) in a portion of the neurons tested in the pelvic plexus ganglia. The blockade of orthodromic action potentials and the depression of f-EPSPs by IL-1ß may be due to inhibition of acetylcholine release from presynaptic terminals.

Methods

Experiments designed to study the effects of bradykinin on mechanosensitive input from distal colon to neurons in pelvic plexus ganglia.

Experiments were performed on adult male guinea-pigs (200-500 gm), euthanized by exsanguination following carbon dioxide gas-induced anesthesia. Pelvic plexus ganglia (PG), pelvic nerves, hypogastric nerves, urethra-urinary bladder nerves, colonic-rectal nerves with an attached segment of distal colon-rectum were dissected *in situ* and placed in a two compartment organ bath (see Fig. 7). The distal colon (6-10 cm in length, originating 2 cm orad from the anus) was placed in one compartment of the organ bath and the PG and attached nerve trunks were placed in the other. The colonic nerves which connect the colon to PG were draped across a 1 mm thick wall separating the two

compartments and covered by moist strips of tissue paper to prevent dehydration. Both bath compartments were superfused separately with a modified Krebs solution containing (in mM): 117 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.5 $NaHCO_3$ and 11 glucose. Solutions were gassed with 95% O_2-5% CO_2 and preheated to 37-38°C at the recording site. In all preparations, pelvic plexus ganglia were pinned onto Sylgard at the bottom of the chamber.

Measurement of intraluminal colonic pressure. The distal colon-rectum was canulated at both ends for distension and measurement of intraluminal colonic/rectal pressure. The catheter at the orad end of the distal colon-rectum was connected to a pressure transducer to continuously monitor intraluminal pressure. The catheter at the aborad end was attached to a Y-valve with one end connected to a calibrated cylindrical reservoir and the other to a syringe. Basal intraluminal pressures (2-6 cm H_2O) were set by the height of Krebs solution (KS) in the calibrated reservoir. Colonic distension was made by switching the position of Y-valve from the reservoir to the syringe and injecting KS into the colon segment.

Drugs was administered by superfusion separately to either the colon-rectal compartment or to the ganglia compartment of the organ bath.

Electrophysiological procedures. Transmembrane potentials were recorded with microelectrode filled with 3 M- KCl. Tip resistance of microelectrodes ranged between 60-110 MΩ. Membrane potential was recorded by conventional microelectrode techniques. An active bridge circuit will allow current injection into neurons through the recording microelectrodes. Signals from the microelectrode was displayed on an oscilloscope with digitized memory and recorded on a pen-writing chart recorder.

Both mechanical and electrical data were stored on a videocassette data recorder and the output (intraluminal pressure, individual electrotonic potentials, f-EPSPs and action potentials) was plotted on a pen-writing chart recorder.

Bradykinin (1-10 nM) was superfused over the colon. Colonic-rectal pressure was monitored continuously during the period of intracellular recording from neurons in pelvic plexus ganglia. The effect of active colon-rectum contraction and passive distension of distal colon-rectum on the electrical activities of PG neurons before and during superfusion of bradykinin were studied.

Experiments designed to test the effects of human recombinant interleukin-1ß on neurons in pelvic plexus ganglia.

Experiments were performed on male guinea-pigs weighing 200-500 gm. Animals were euthanized by exsanguination following a sharp blow to the head. Pelvic plexus ganglia with attached central (pelvic and hypogastric) and peripheral (urethra-urinary bladder) nerves were dissected *in situ* and placed in an organ bath. Pelvic plexus ganglia were pinned onto Sylgard at the bottom of a chamber (0.5 ml) and continuously superfused with a Krebs solution (2-3 ml/min) containing (in mM): 117 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 25 $NaHCO_3$ and 11 glucose. Solutions were gassed with 95% O_2-5% CO_2 and preheated to 36-37 °C at the recording site.

Microelectrodes were filled with 3 M KCl. The tip resistances of microelectrodes ranged from 25 to 60 MΩ. Membrane potentials were recorded by conventional microelectrode techniques. An active bridge circuit allowed current injection into neurons through a recording microelectrode. Membrane current was recorded by a discontinuous single-electrode voltage clamp method with an Axoclamp 2A (Axon Instruments) amplifier. Sampling frequency ranged between 3 and 5 kHz with a 70-30 duty cycle. The reversal potentials were estimated from I/V plots using a least squares linear regression analysis. Signals from the microelectrode were displayed on an oscilloscope, recorded on a pen-writing chart recorder and stored on a videocassette data recorder.

Fast excitatory postsynaptic potentials (f-EPSPs) and associated action potentials were evoked by electrical stimulation of fibers in pelvic, hypogastric or urethra-urinary bladder nerves (0.3 - 0.5 ms duration, frequency 0.3 - 0.5 Hz). Acetylcholine (100 - 200 μM in Krebs solution) was pressure ejected (20 psi for 10 - 250 ms) onto the surface of neurons from a micropipette (tip diameter ~ 5 - 10 μm). Pressure application of Ach caused a fast membrane depolarization (fast Ach potential) and slow membrane depolarization (slow Ach potential). Fast Ach potentials were blocked by hexamethonium (10 - 100 μM), indicating that they are mediated by nicotinic receptors. Slow Ach potentials were abolished by atropine (300 nM), indicating that they are mediated by muscarinic receptors. Membrane input resistance was determined by measuring the steady state voltage evoked by injection of hyperpolarizing current across the cell membrane (30 - 200 ms duration) and calculated as the ratio between the magnitude of steady state voltage and hyperpolarizing current. Data are expressed as mean ± SE.

Human recombinant interleukin-1ß (hrIL-1ß, 1 μg/ml in 0.1% of bovine serum albumin) was dissolved in Dulbecco's phosphate buffer sterile solution. It was applied by either superfusing the ganglia with a Krebs solution containing hrIL-1ß (20 - 80 ng/ml) or by pressure ejection (1 μg/ml, 20 psi for 200 - 1200 ms) onto the surface of neurons. Other drugs were dissolved directly into the Krebs solution and applied by superfusion. A specific IL-1 receptor antagonist was used to test the specificity of hrIL-1ß evoked responses. Bovine serum albumin (BSA, 0.1%) had no effect on active and passive membrane properties of neurons or on synaptic transmission.

Drugs used were human recombinant interleukin-1ß (hrIL-1ß) (R & D system, Inc.), human recombinant interleukin-1 receptor antagonist (hrIL-1ra) (Synergen Inc.), bovine serum albumin (BSA), tetraethylammonium chloride (TEA), tetrodotoxin (TTX), ω-conotoxin GVIA, yohimbine hydrochloride, hexamethonium bromide, atropine sulfate, acetylcholine chloride and naloxone hydrochloride (Sigma).

Data were expressed as mean ± SEM.

Results

Effects of bradykinin on colon contraction and mechanoreceptor mediated synaptic input to neurons in pelvic plexus ganglia.

Superfusion of the distal colon segment with Krebs solution containing bradykinin (1-10 nM, 20 min) induced a tonic contraction. Associated with the colon contraction, f-EPSPs and action potentials were initiated or the frequency of spontaneous f-EPSPs and action potentials and the amplitude of f-EPSPs were increased in pelvic plexus ganglion neurons in electrically quiescent (2 of 12 preparations) or with minimum spontaneous electrical activities (2 of 12 preparations) (Fig. 32). The remainder neurons had no response to BK application. In the four neurons that responded to colon application of BK, passive distension of the colon-rectum initiated or increased electrical activities (Fig. 33) while before the application of bradykinin, passive colonic distension did not increase electrical activities in these neurons. These data suggest that bradykinin sensitized the mechanosensory input from distal colon to neurons in pelvic plexus ganglia. This effect may have been due to a direct action of bradykinin on mechanosensory terminals of peripheral afferent fibers as well as an indirect activation of mechanoreceptors by stimulation of smooth muscle contraction. Also, the existence of polymodal receptors which are sensitive both to mechanical stimuli and chemostimuli, can not be excluded. When bradykinin (10 nM) was superfused on pelvic plexus ganglia only (n=3), it had no effect on membrane potential and membrane input resistance of the neurons, indicating that increased electrical activities of pelvic plexus ganglion neurons was not due to a direct effect of bradykinin on these neurons. The lack of response of pelvic plexus ganglion neurons to BK may be due to the lack of BK receptors on these neurons.

Figure 32. Effects of bradykinin (10 nM) on spontaneous colon contractions and electrical activities of one neuron in pelvic plexus ganglia. In each panel, top traces represent intracolonic pressures and bottom traces represent electrical activities recorded from one neuron in pelvic plexus ganglia. A, Intracolonic pressure and electrical activities of a neuron when the colon was superfused with a normal Krebs Solution (KS). B, C, Intracolonic pressure and electrical activities of the same neuron during superfusion of the colon with a Krebs solution containing bradykinin (10 nM).

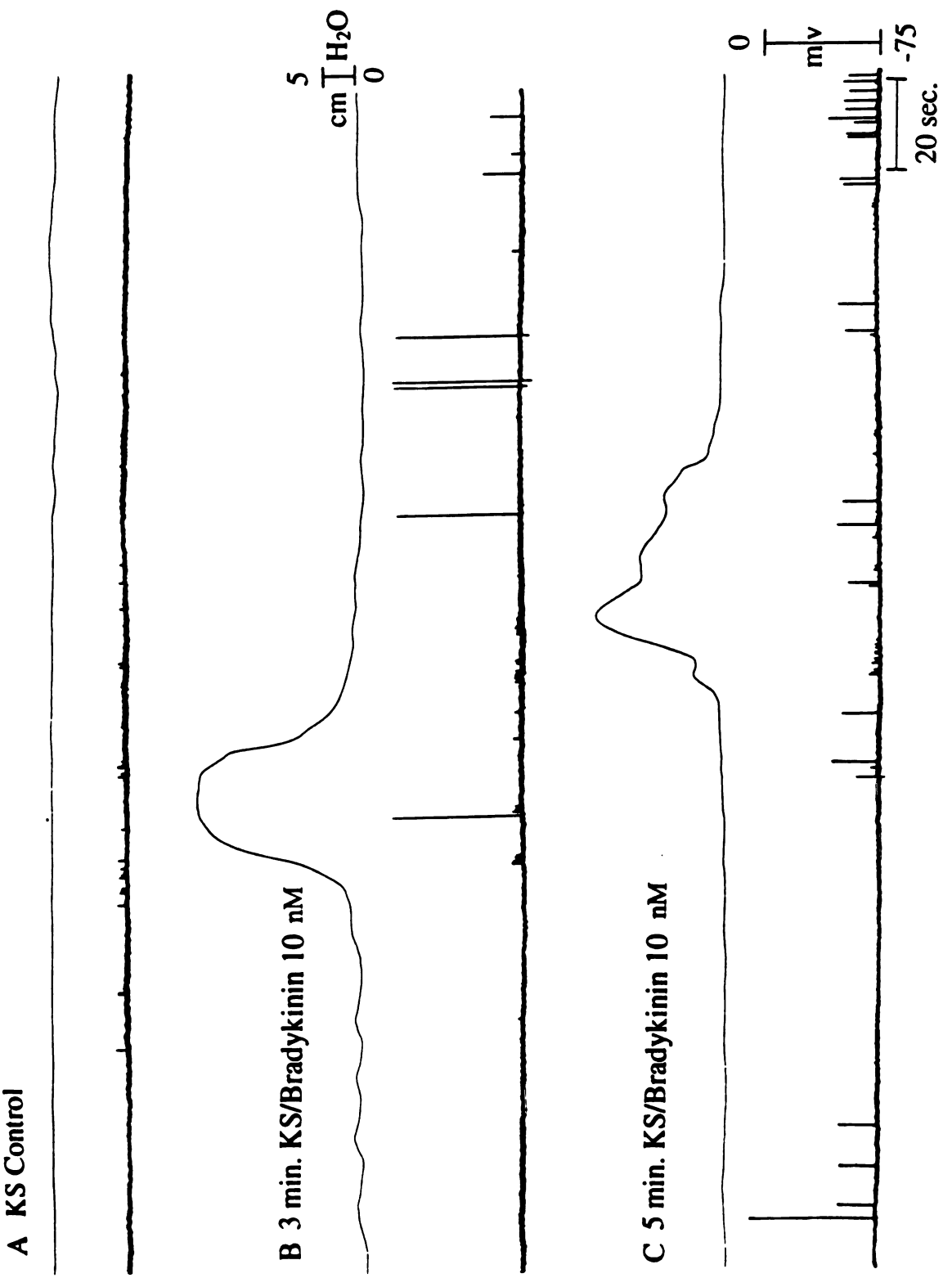
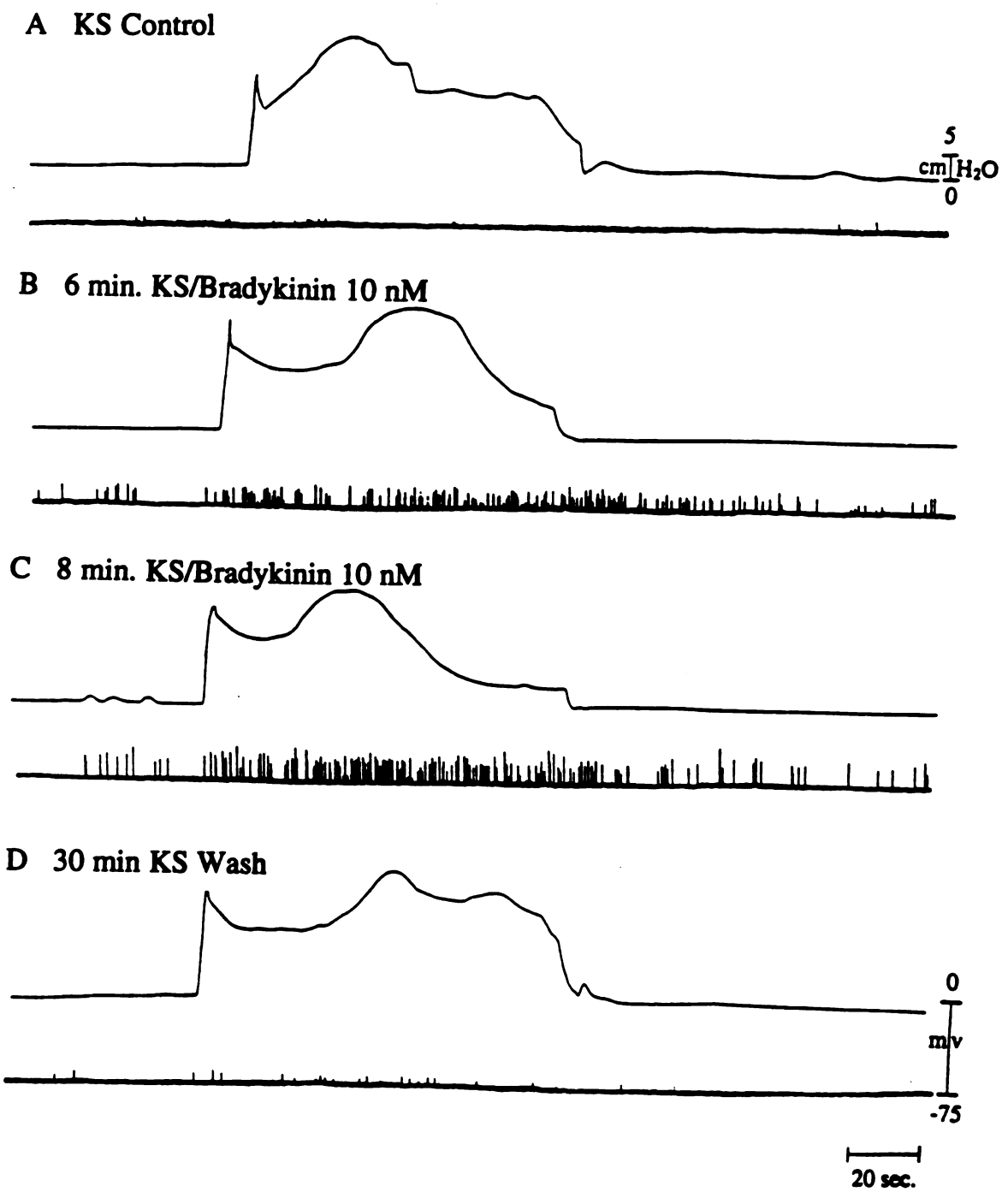

A KS Control
B 3 min. KS/Bradykinin 10 nM
C 5 min. KS/Bradykinin 10 nM
5
cm H2O
0
0
mv
-75
20 sec.

Figure 33. Effect of colon-rectum distension on synaptic inputs (fast excitatory postsynaptic potentials) of a neuron in pelvic plexus ganglia before (A), during (B, C) and after (D) bradykinin (10 nM) was superfused on colon-rectum. A, effect of colon-rectum distension on synaptic potentials when colon-rectum and pelvic plexus ganglia were superfused with a normal Krebs solution. B and C, effect of colon-rectum distension on synaptic potentials of the same neuron when colon-rectum was superfused with a Krebs solution containing bradykinin (10 nM) for 6 min and 8 min, respectively. D, effect of colon-rectum distension on synaptic potentials in the same neuron when colon-rectum was superfused with a normal Krebs solution for 30 min.


A KS Control
5
cm H2O
0
B 6 min. KS/Bradykinin 10 nM
C 8 min. KS/Bradykinin 10 nM
D 30 min KS Wash
0
mv
-75
20 sec.

Effects of human recombinant interleukin-1ß on membrane potential and membrane input resistance

Pressure or bath application of human recombinant interleukin-1ß (hrIL-1ß) caused membrane depolarization (54%; 36 of 66) and membrane hyperpolarization (30%; 20 of 66) of neurons tested in guinea-pig pelvic plexus ganglia. HrIL-1ß had no effect on the remainder of cells tested.

Depolarization In 28 of the neurons responding with membrane depolarization, hrIL-1ß (1 µg/ml) was applied by pressure injection (200-1200 ms, 20 psi). In these neurons, the membrane depolarization was associated with a decrease in membrane input resistance (46.0 ± 4.5%) (Fig. 34A, B). HrIL-1ß-evoked depolarizations (1 µg/ml, 20 psi, 900 ms) had a mean time to peak of 1.2 ± 0.2 min, a mean peak amplitude of 11.8 ± 1.9 mV and a mean duration of 4.6 ± 0.9 min. The effect of pressure-injected IL-1ß was dose-dependent (Fig. 35A). Pressure application of hrIL-1ß (1 µg/ml, 600 ms duration, 20 psi, at holding potentials of -56 to -69 mV) also caused inward currents with a mean time to peak, peak amplitude and duration of 0.9 ± 0.2 min, 440 ± 72 pA and 4.1 ± 1.5 min, respectively (Figure 34C). In 8 of the neurons responding with membrane depolarization, hrIL-1ß was applied by superfusion (20 - 80 ng/ml; 5 min.). At a concentration of 40 ng/ml, membrane depolarizations had a mean time to peak of 2.7 ± 0.7 min, a mean peak amplitude of 14.2 ± 2.9 mV and a mean duration of 9.6 ± 2.8 min.

Hyperpolarization Pressure application of hrIL-1ß (1 µg/ml, 600 ms duration, 20 psi) evoked membrane hyperpolarization with a mean time to peak, peak amplitude and duration of 22.1 ± 8.8 sec, 8.2 ± 0.9 mV and 1.2 ± 0.3 min, respectively. HrIL-1ß-evoked membrane hyperpolarization was associated with an increase in membrane input resistance (31.1 ± 1.2%) (Fig. 36A, B). The hyperpolarization of membrane potential by hrIL-1ß was dose dependent (Fig. 35B). Pressure application of hrIL-1ß (1 µg/ml, 500 ms duration, 20 psi, at holding potentials of -48 to -72 mV) also caused an outward membrane current with a mean time to peak, peak amplitude and duration of 18.3 ± 3.3

Figure 34. Effects of human recombinant interleukin-1ß (hrIL-1ß) on resting membrane potential, membrane input resistance and membrane current of two neurons in pelvic plexus ganglia. A: continuous recording of membrane potential from one neuron. Top and bottom traces, membrane potential and injected current through a microelectrode, respectively. Resting membrane potential was -60 mV. HrIL-1ß was pressure ejected (1 µg/ml, 300 ms duration, 20 psi.). Membrane input resistance, calculated as the ratio between the amplitude of steady state voltage of hyperpolarizing pulse and current injected into the cell decreased (b, c, d) during the hrIL-1ß-evoked membrane depolarization. B: expanded records of hyperpolarizing electronic potentials. Records a-e were obtained at the time marked by respective letters in A. C: continuous recording of membrane current from another neuron. Inward membrane current was evoked by pressure ejection of hrIL-1ß (1 µg/ml, 600 ms duration, 20 psi.). Holding potential was -58 mV (resting membrane potential).

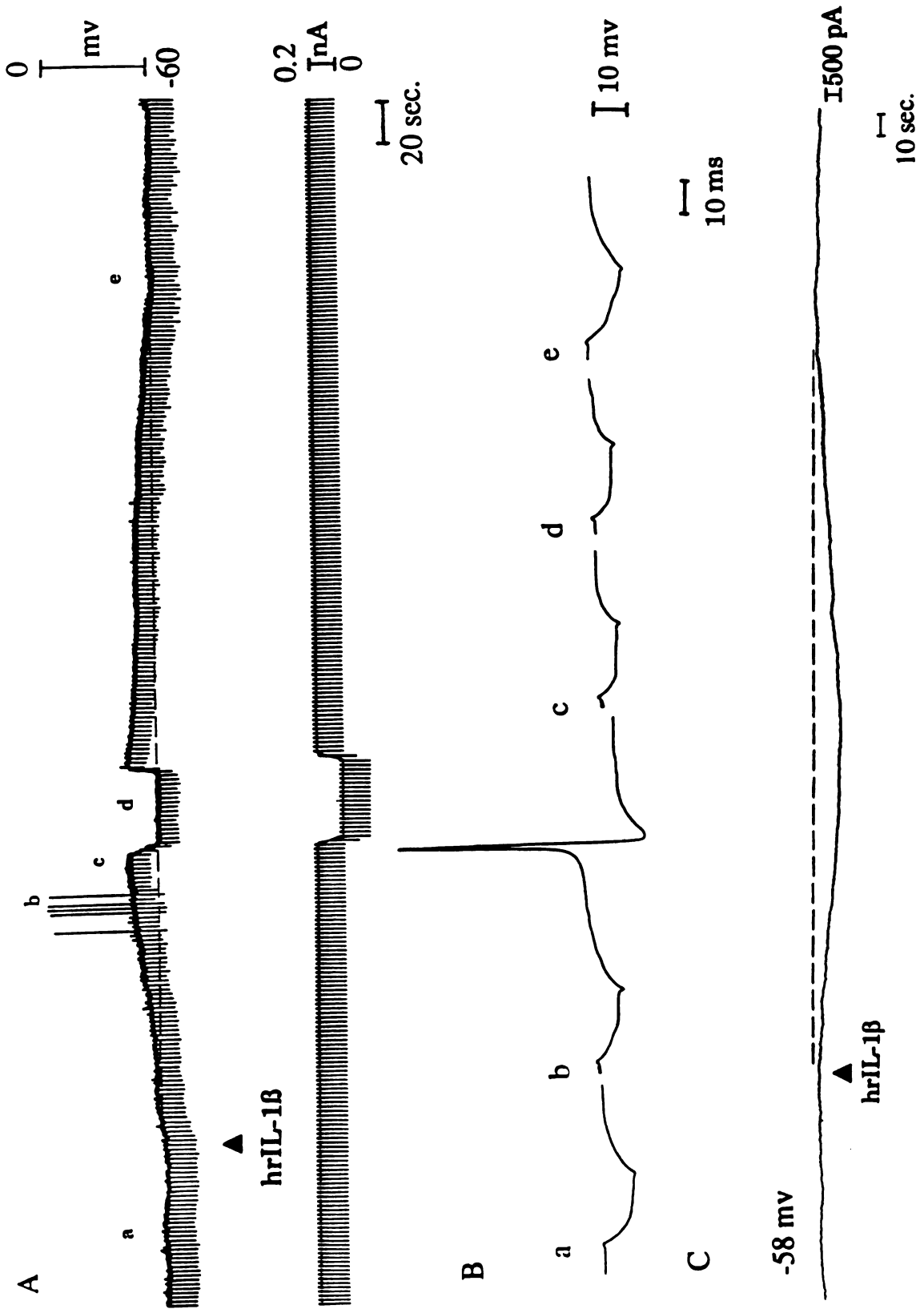

A
0
mv
-60
a
b
c
d
e
hrIL-1ß
0.2
InA
0
20 sec.
B
a
b
c
d
e
10 mv
C
10 ms
-58 mv
500 pA
10 sec.
hrIL-1β

Figure 35. Effects of human recombinant interleukin-1ß (hrIL-1ß) on resting membrane potential. Ordinate, change in membrane potential amplitude. Abscissa, duration of pressure ejection of hrIL-1ß (1 µg/ml, 20 psi.), expressed in ms. A: depolarization effect of hrIL-1ß. B: hyperpolarization effect of hrIL-1ß. Vertical bars and number above symbols are standard error of mean and number of cells tested, respectively.

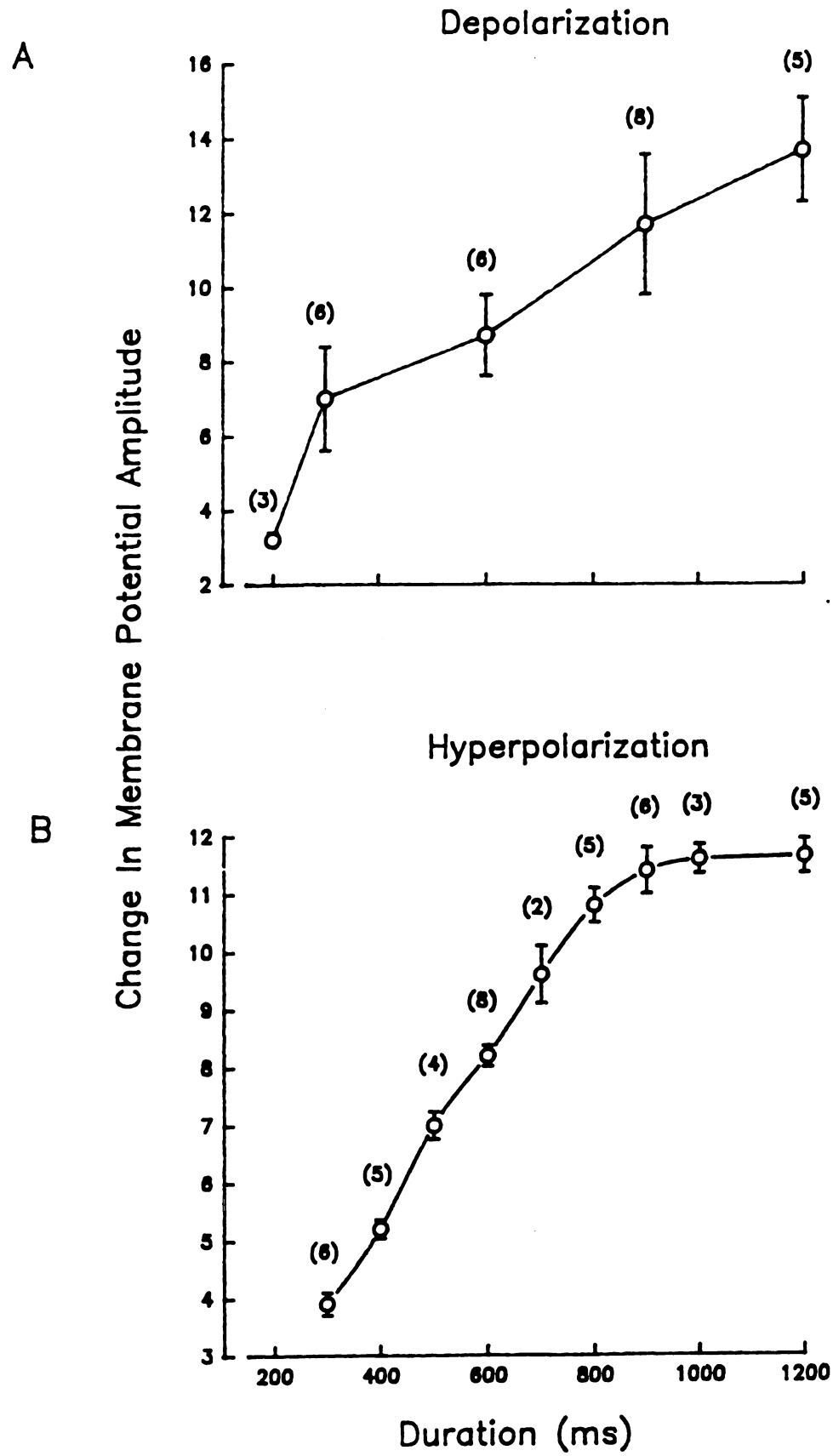
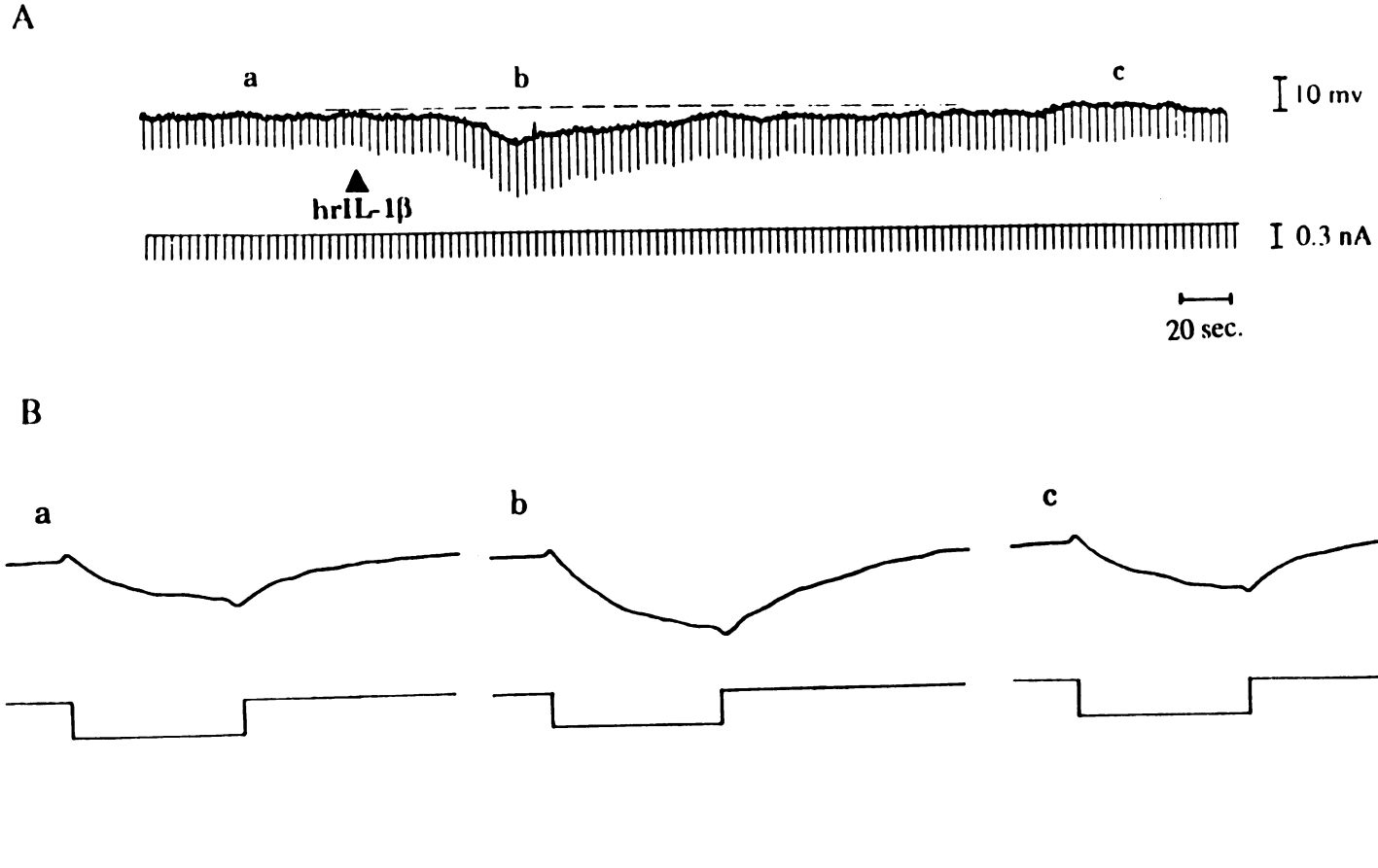

A
Depolarization
16
14
12
10
8
6
4
2
(3)
(6)
(6)
(8)
(5)
B
Hyperpolarization
12
11
10
9
8
7
6
5
4
3
(6)
(5)
(4)
(8)
(2)
(5)
(6)
(3)
(5)
200
400
600
800
1000
1200
Change In Membrane Potential Amplitude
Duration (ms)

Figure 36. Effects of human recombinant interleukin-1ß (hrIL-1ß) on resting membrane potential, membrane input resistance and membrane current of two neurons in pelvic plexus ganglia. A: continuous recording of membrane potential from one neuron. Top and bottom traces, membrane potential and injected current through a microelectrode, respectively. Resting membrane potential was -66 mV. HrIL-1ß was pressure ejected (1 µg/ml, 600 ms duration, 20 psi.). Membrane input resistance was calculated as the ratio between the amplitude of steady state voltage of hyperpolarizing pulse and current injected into the cell. Membrane input resistance increased (b) during the hrIL-1ß-evoked hyperpolarization. B: expanded records of hyperpolarizing electronic potentials. Records a-c were obtained at the time marked by respective letters in A. C: continuous recording of membrane current from another neuron. Outward membrane current was evoked by pressure ejection of hrIL-1ß (1 µg/ml, 600 ms duration, 20 psi.). Holding potential was -64 mV (resting membrane potential).


A
a
b
c
10 mv
hrIL-1β
0.3 nA
20 sec.
B
a
b
c
10 mv
0.3 nA
10 ms
C
-64 mV
500 pA
10 sec.
hrIL-1β

sec, 0.7 ± 0.2 nA and 1.0 ± 0.5 min respectively (Fig. 36 C). The voltage sensitivity of hrIL-1β-evoked outward currents was tested by clamping the neuron to various potentials ranging from -10 to -100 mV and measuring the amplitude of the current. HrIL-1β-induced outward current was decreased by depolarization and increased by hyperpolarization of the membrane potential (Fig. 37). The extrapolated reversal potential was -5 ± 4.5 mV (n=3).

Membrane potential, depolarization, hyperpolarization and associated changes in membrane input resistance evoked by hrIL-1ß were not altered by hexamethonium (100 μM), atropine (500 nM), yohimbine (300 nM), naloxone (1 μM), ω-conotoxin (GVIA, 300 - 700 nM), tetrodotoxin (TTX, 1 - 5 μM) or tetraethylammonium (TEA, 30 - 80 mM) (n=3 for each agent) (Fig. 38).

Tachyphylaxis to the hrIL-1ß-evoked membrane depolarization occurred after the first bath (20 - 80 ng/ml, 3 - 6 min) or pressure application (1 μg/ml, 200 - 1200 ms, 20 psi). Subsequent membrane responses to hr-IL-1ß were either smaller in magnitude or there were no effects (n=28). Tachyphylaxis was more pronounced for the hr-IL-1ß-evoked membrane depolarization than membrane hyperpolarization.

Effect of hrIL-1ß on Synaptic Transmission

Orthodromic action potentials and fast excitatory postsynaptic potentials (f-EPSPs). Stimulation of fibers in pelvic, hypogastric and urethra-urinary bladder nerves at supramaximal intensities evoked f-EPSPs and orthodromic action potentials that were reversibly blocked by superfusing the ganglia with a low calcium (0.05 mM), high magnesium (30 mM) Krebs solution (n=3) or a Krebs solution containing hexamethonium (10 - 100 μM, n=3) or dihydro-ß-erythroidine (5 - 100 μM, n=3). In contrast, atropine (300 - 500 nM) did not alter orthodromic action potentials and f-EPSPs. This indicates that the responses were mediated via nicotinic acetylcholine receptors.

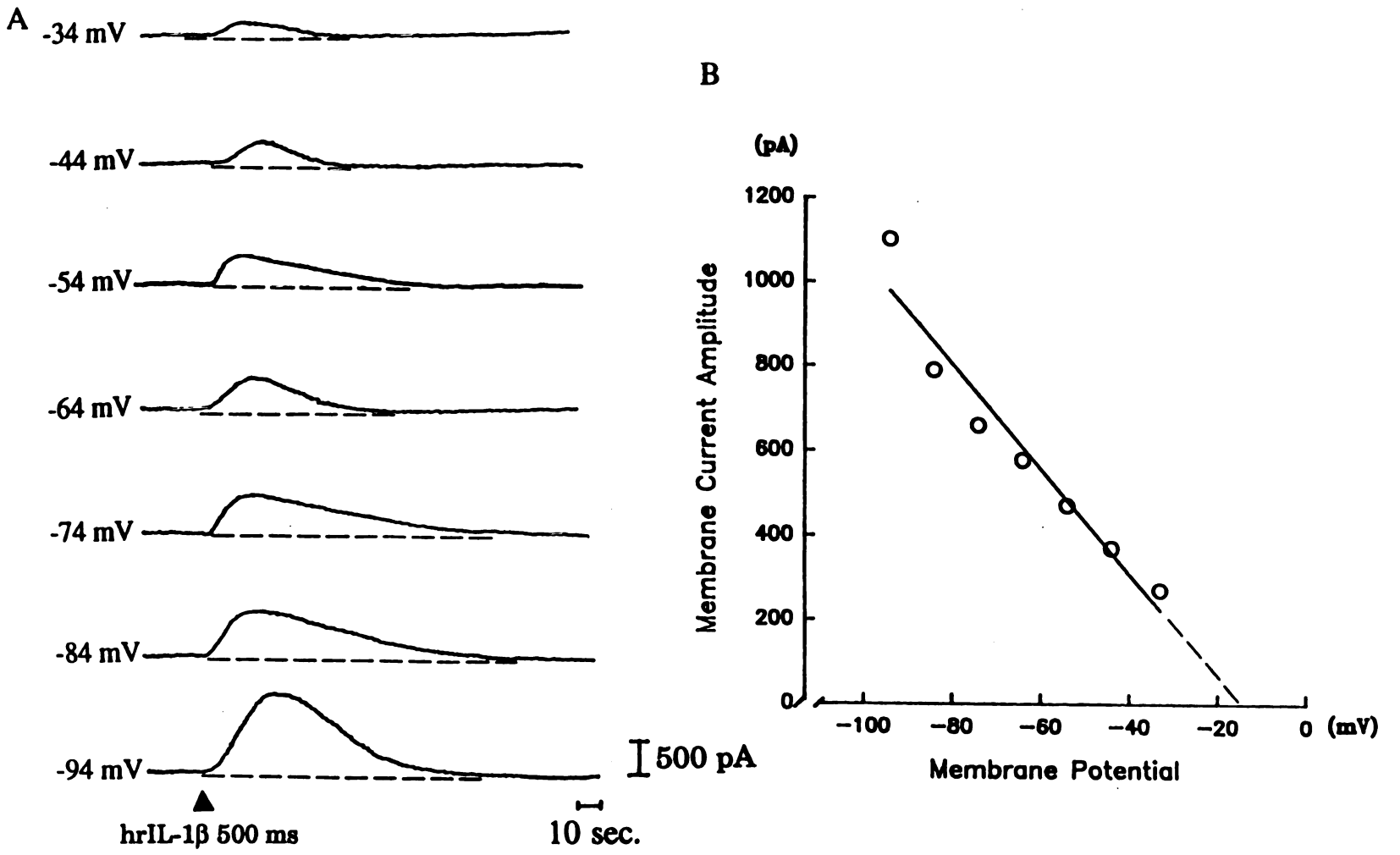
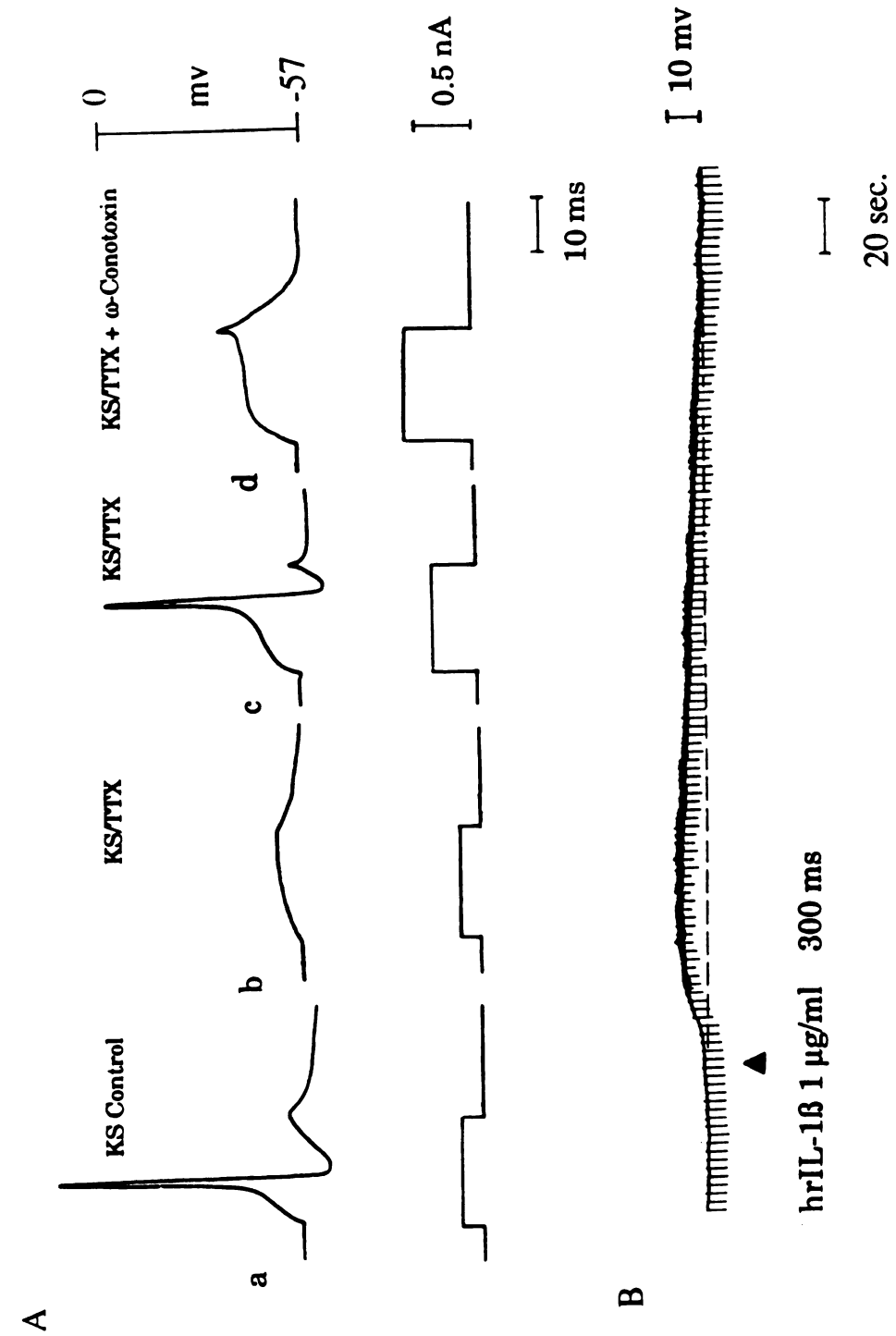


Figure 37. Human recombinant interleukin-1ß (hrIL-1ß) induced outward currents at different holding potentials (A) and voltage sensitivity of these currents (B) recorded from a neuron in pelvic plexus ganglia. HrIL-1ß (1 µg/ml) was ejected by a constant pressure pulse (20 psi, 500 ms). The extrapolated reversal potential was -15 mV.

Figure 38. A: Effects of tetrodotoxin (TTX) and ω-conotoxin on action potentials evoked by direct depolarizing current injection (20 ms, 0.2-0.6 nA). a, control action potential. b, application of TTX (5 μM) abolished action potential discharge. c, in the presence of TTX (5 μM), an action potential with lower amplitude and slower rate of rise was achieved by increasing the intensity of depolarizing pulse. d, superfusion of ω-conotoxin (700 nM) abolished TTX resistant action potential. B: Effects of human recombinant interleukin-1ß (1 μg/ml, 300 ms) on membrane potential and input resistance of the same neuron in the presence of both TTX (5 μM) and ω-conotoxin (700 nM).


A
KS Control
KS/TTX
KS/TTX + ω-Conotoxin
0
mv
-57
a
b
c
d
0.5 nA
10 ms
B
10 mv
hrIL-1ß 1 µg/ml 300 ms
20 sec.

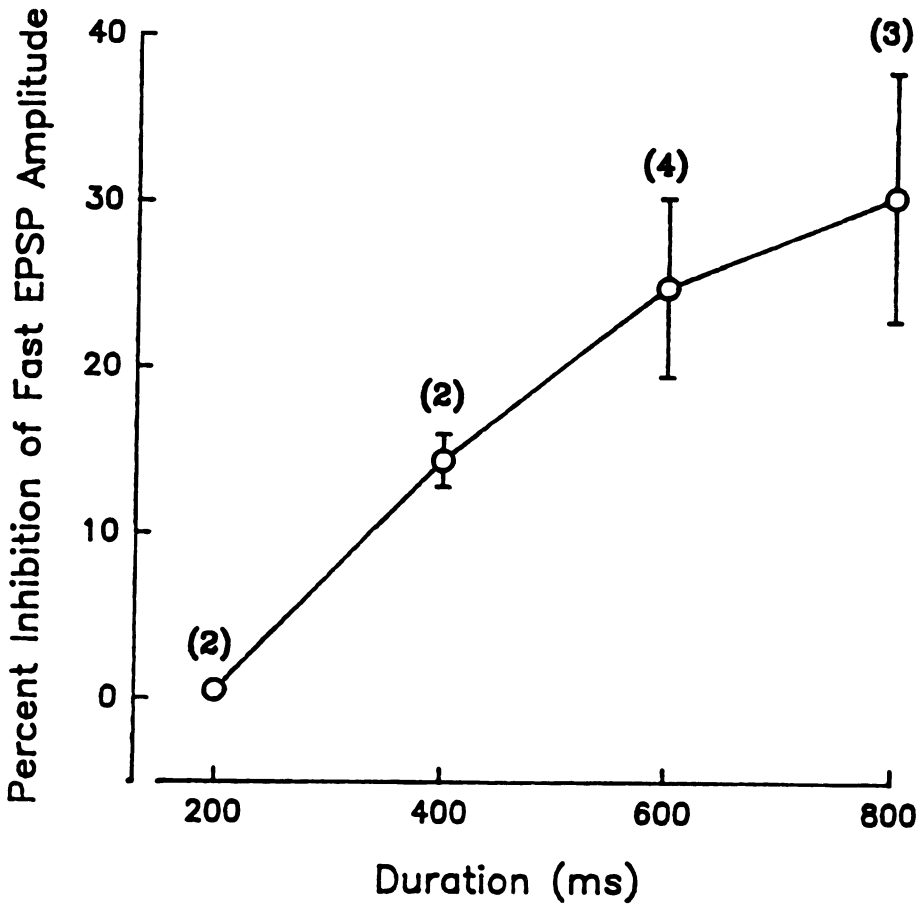


Figure 39. Effect of human recombinant interleukin-1ß (hrIL-1ß) on amplitude of fast excitatory postsynaptic potentials (f-EPSPs). Ordinate, percent inhibition of f-EPSP amplitude. Abscissa, duration of pressure ejection of hrIL-1ß (1 µg/ml, 20 psi.), expressed in ms. Vertical bars and number above symbols are standard error of mean and number of cells tested, respectively.

Pressure application of hrIL-1ß (1 µg/ml, 400 - 1200 ms. 20 psi) caused a depression of f-EPSPs and blockage of orthodromic action potentials in 44 % (7 of 16) of neurons tested. When nerve fibers were stimulated at intensities that initiated only f-EPSPs, the maximum depression of f-EPSPs amplitude by hrIL-1ß (1 µg/ml, 800 ms, 20 psi) was 30.3 ± 7.5 %. The action of hrIL-1ß on the amplitude of f-EPSPs occurred in a dose-dependent manner (Fig. 39).

Bath application of hrIL-1ß (40 - 80 ng/ml) also caused a depression of f-EPSPs amplitude in 42% (3 of 7) of neurons tested (Fig. 40). The maximum depression of f-EPSPs amplitude caused by hrIL-1ß (80 ng/ml) was 88.5 ± 9.7%. The depression of f-EPSPs by hrIL-1ß also occurred when there was no change in transmembrane potential (n=2) or when hrIL-1ß-evoked depolarizations (n=4) or hyperpolarizations (n=3) were nullified by injecting hyperpolarizing or depolarizing current through the microelectrode, respectively (Fig. 40).

Effect of hrIL-1ß on acetylcholine potentials mediated by nicotinic receptors. HrIL-1ß may depress the amplitude of f-EPSPs by altering the sensitivity of the postsynaptic cholinoceptors to endogenously released Ach. To test this possibility, we examined the effects of hrIL-1ß on membrane potential changes caused by exogenous Ach. The effect of hrIL-1ß (40 - 80 ng/ml) was tested on nicotinic fast Ach potentials (n =3). The Krebs solution contained tetrodotoxin (300 nM) to block action potentials that are associated with fast Ach potentials and atropine (300 nM) to block slow Ach potentials mediated by muscarinic receptors. Figure 41 shows that HrIL-1ß (80 ng/ml) caused membrane depolarization associated with a decrease in the amplitude of fast Ach potentials recorded from one neuron. After the hrIL-1ß-evoked membrane depolarization was nullified by direct injection of hyperpolarizing current, the amplitude of fast Ach potentials was not changed.

Specificity of hrIL-1ß effects

IL-1 receptor antagonist (IL-1ra) binds to IL-1 receptors and inhibits the binding of IL-1ß (Dinarello and Thompson, 1991). Superfusion of IL-1ra (10-40 μg/ml, 5 -10 min) alone did not alter the passive and active membrane properties and synaptic transmission of neurons in pelvic plexus ganglia (n=7). However, it blocked the effects of hrIL-1ß on membrane depolarization (n=3), hyperpolarization (n=3) (Fig. 42) and synaptic transmission of neurons in pelvic plexus ganglia (n=2) (Fig. 43). IL-1ra (20 μg/ml and 40 μg/ml) caused a concentration-dependent suppression of the hrIL-1ß-evoked membrane hyperpolarization by 65.2 ± 3.2% and 96.0 ± 4.6%, respectively. The data indicate that the effects of hrIL-1ß on resting membrane potential and on synaptic transmission are mediated by specific interaction with IL-1 receptors.

Discussion

The effect of bradykinin on mechanosensory input(s) to neurons in pelvic plexus ganglia.

The data show that application of bradykinin to the distal colon initiated or increased the mechanosensory inputs to a portion of neurons in the pelvic plexus ganglia. The effects was associated with bradykinin-evoked contractions of colon. Bradykinin had no effects on electrical activities and membrane properties of neurons in pelvic plexus ganglia, when applied only to the ganglia site. The data indicate that bradykinin sensitizes the mechnosensory input to neurons in pelvic plexus ganglia from peripheral organs. The actions of bradykinin may be due to direct depolarization of afferent terminals by activating the silent mechanoreceptors that only can be activated during inflammation. Another possibility is that bradykinin interacts with a certain type of polymodal receptors, which can be activated by both mechanical and chemical stimuli (Haupt, Jänig and Kohler, 1983; Jänig and Koltzenberg, 1991). The actions of bradykinin

Figure 40. Effects of human recombinant interleukin-1ß (hrIL-1ß) on fast excitatory postsynaptic potentials (f-EPSPs) of one neuron in pelvic plexus ganglia. Horizontal lines, time period for superfusion of hrIL-1ß (80 ng/ml). Resting membrane potential was -56 mV. A: continuous recordings of membrane potential and f-EPSPs (upward deflections) evoked by pelvic nerve stimulation. HrIL-1ß reduced the amplitude of f-EPSPs during depolarization and when membrane potential was at the resting level (b). During time period between filled circles, hrIL-1ß-evoked depolarization was nullified by injection of anodal direct current. B: trace obtained 5 min. after removal of hrIL-1ß. C: continuous recording of B. Second application of hrIL-1ß did not evoke membrane depolarization but reduced the amplitude of f-EPSPs. D: expanded records of f-EPSPs at the time marked by respective letters in traces A, B and C. In each record, 5 consecutive responses are superimposed.

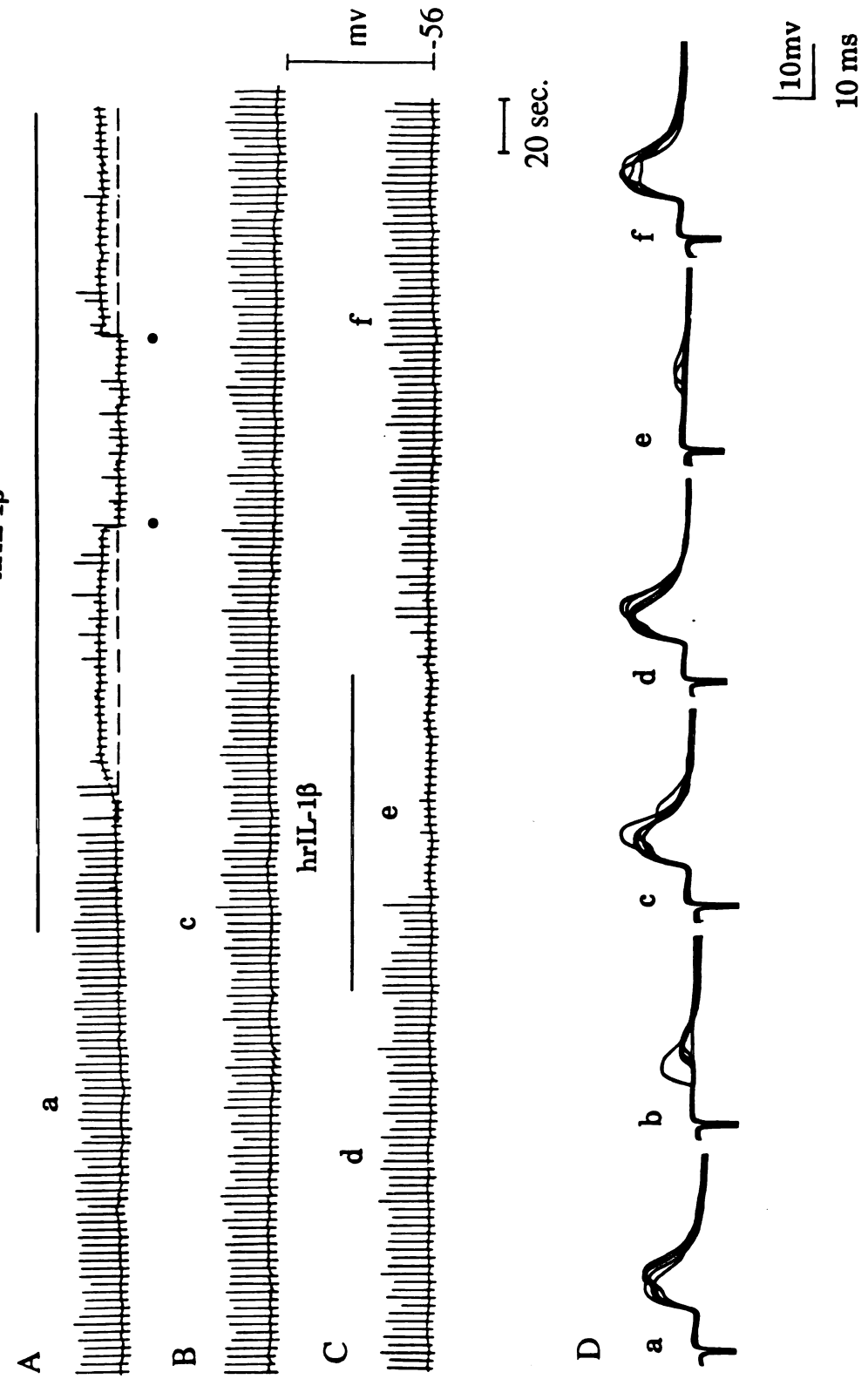
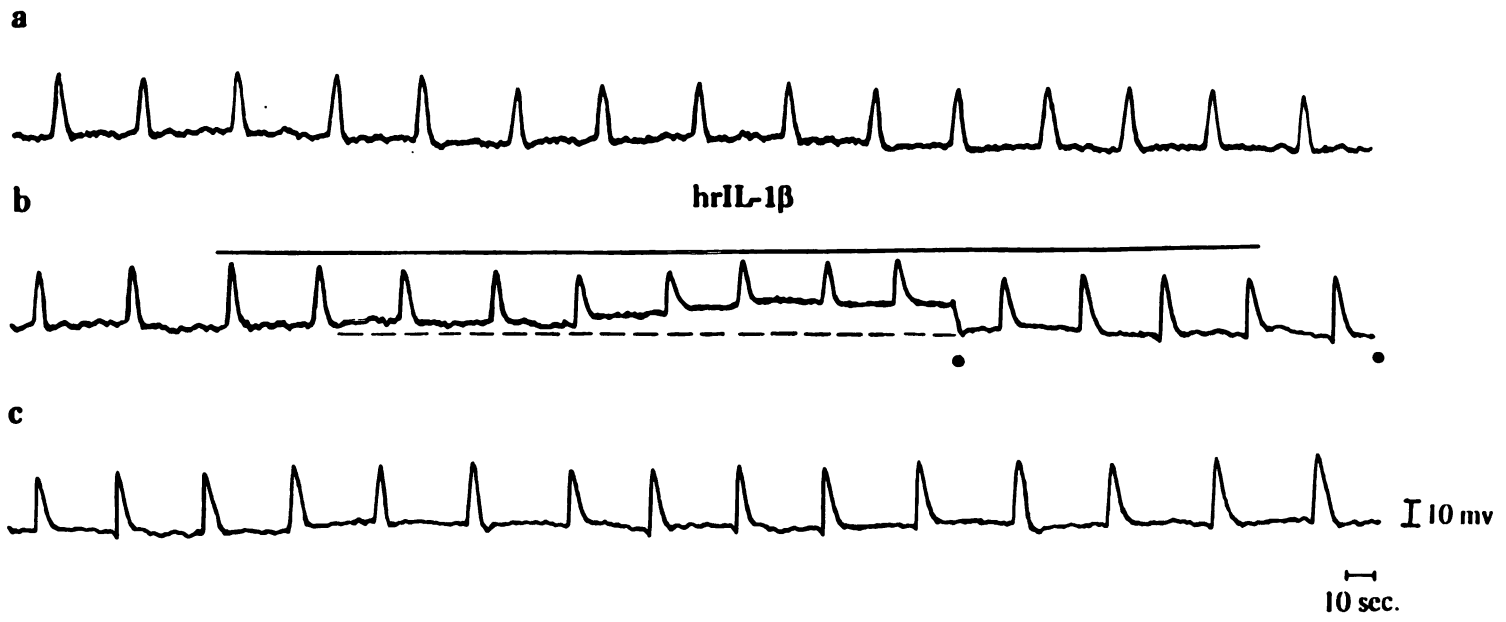

A
a
hrIL-1β
B
c
C
d
e
hrIL-1β
f
mv
-56
20 sec.
D
a
b
c
d
e
f
10mv
10 ms

Figure 41. Effect of human recombinant interleukin-1ß (hrIL-1ß) on fast acetylcholine (Ach) potentials of one neuron in pelvic plexus ganglia. A: fast Ach potentials were recorded in Krebs solution containing tetrodotoxin (300 nM) and atropine (300 nM). Horizontal line, time period of application of hrIL-1ß (80 ng/ml). During time period between filled circles, hrIL-1ß-evoked depolarization was nullified by injection of anodal direct current. Resting membrane potential was -57 mV. Traces a and b, continuous recording of fast Ach potentials. Trace c, trace obtained 5 min. after b. B: amplitude of successive fast Ach potentials for neuron in A before (○), during (●) and after (○) application of hrIL-1ß. Note, amplitude of Ach potential was similar to the control when hrIL-1ß-induced depolarization was nullified.

A


a
b
hrIL-1β
c
10 mv
10 sec.

B

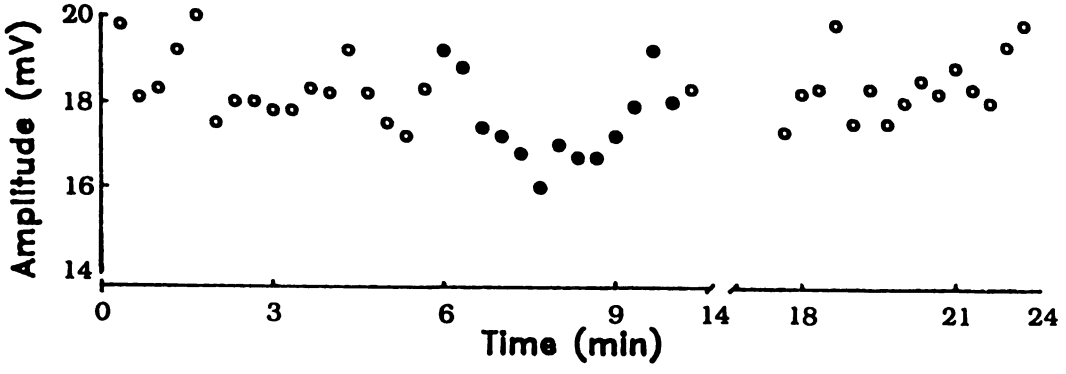

Amplitude (mV)
20
18
16
14
0
3
6
9
14
18
21
24
Time (min)

Figure 42. Effects of IL-1 receptor antagonist (IL-1ra) on pressure application of human interleukin-1ß (hrIL-1ß) induced membrane hyperpolarization in one neuron. Top traces: hrIL-1ß (1μg/ml, 20 psi, 900 ms) caused membrane hyperpolarization when pelvic plexus ganglia were superfused with a normal Krebs solution. Middle traces: hrIL-1ß (1μg/ml, 20 psi, 900 ms) caused membrane hyperpolarization 6 min. after pelvic plexus ganglia were superfused with a Krebs solution containing IL-1ra 20 μg/ml (a) and IL-1ra 40 μg/ml (b). Bottom traces: hrIL-1ß (1μg/ml, 20 psi, 900 ms) caused membrane hyperpolarization 10 min. after pelvic plexus ganglia were superfused with a normal Krebs solution again. Membrane potential was -58 mV.

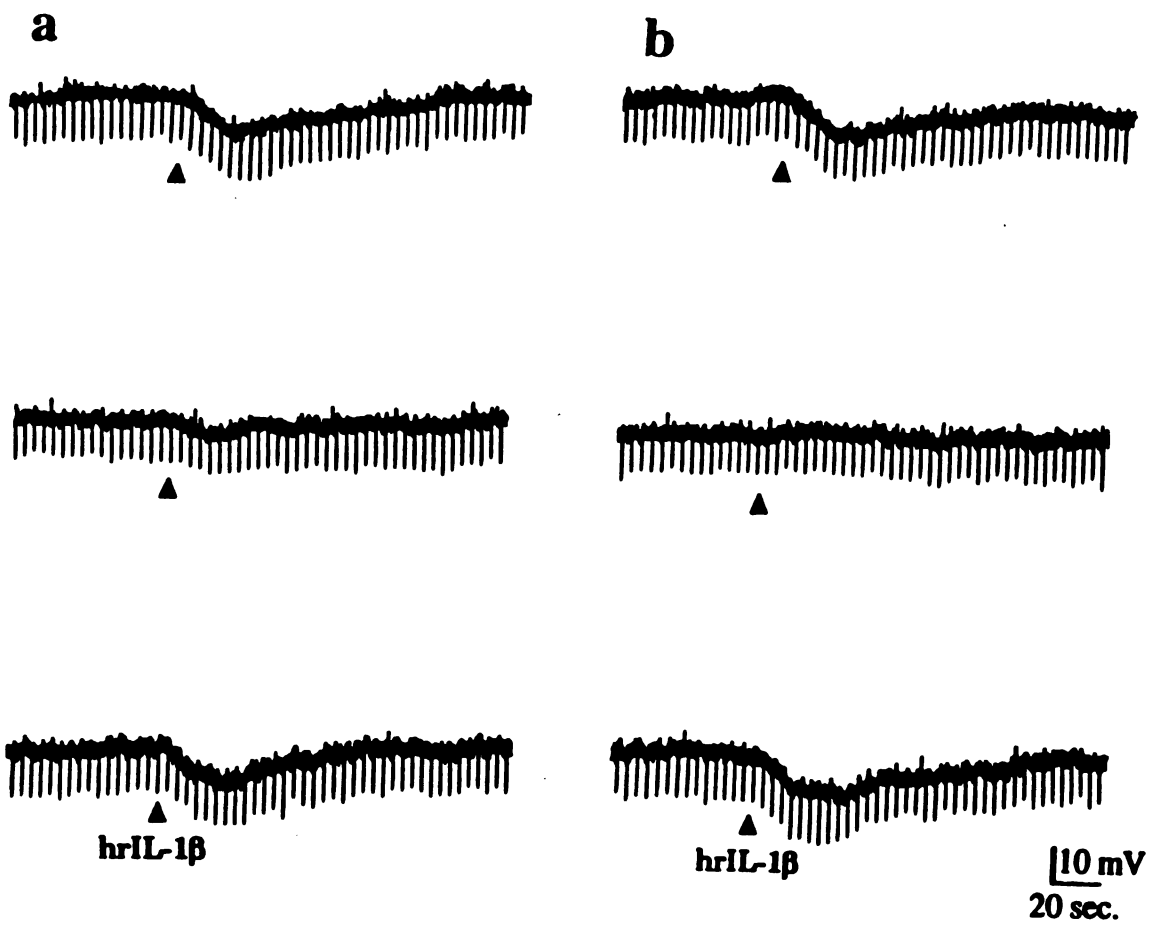
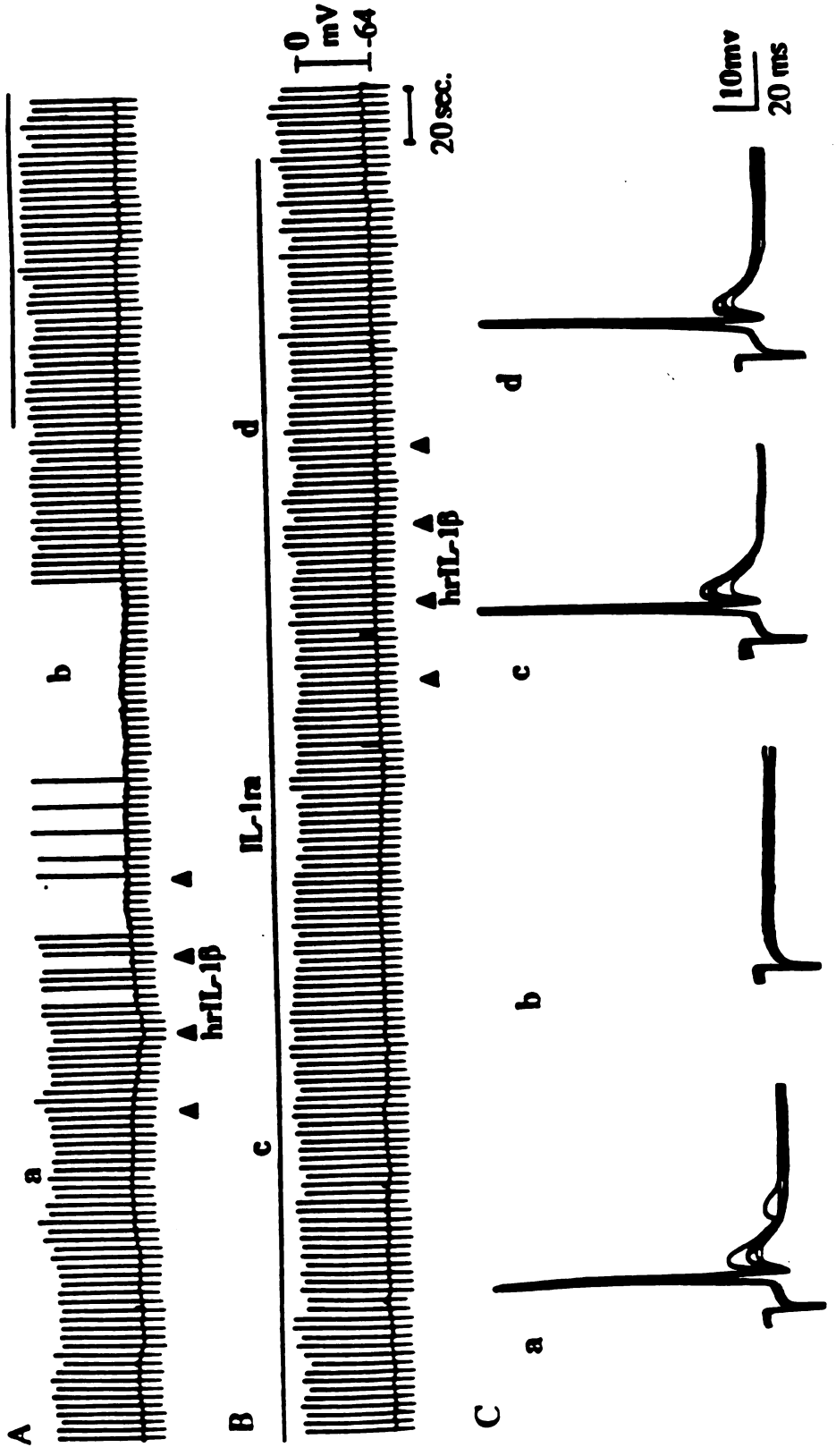

a
b
hrIL-1β
hrIL-1β
10 mV
20 sec.

Figure 43. Effects of human interleukin-1ß (hrIL-1ß) on synaptic potentials of one neuron in pelvic plexus ganglia in the absence and presence of IL-1 receptor antagonist (IL-1ra). A and B: continuous recordings of membrane potential and synaptic potentials evoked by electrical stimulation of urethra-urinary bladder nerves (large upward deflections) before and after pressure application of hrIL-1ß (1 μg/ml, 20 psi, 300 ms × 4, 30 sec. interval) in the absence (A) and presence (B) of IL-1ra 40 μg/ml. Horizontal line, time period for superfusion of IL-1ra. Membrane potential was -64 mV. C: expanded records of synaptic potentials at the time marked by respective letters in traces A and B. In each record, 5 consecutive responses were superimposed.


A
B
C
a
b
c
d
hrIL-1β
IL-1α
0
mV
-64
20 sec.
10mv
20 ms

may also be due to activation of mechanoreceptors by the smooth muscle contraction caused by bradykinin.

Bradykinin is released during inflammation and plays an important role in mediating hyperalgesia and hyperreflexia through the central nervous system by activating on visceral primary afferents (Keele and Armstrong, 1964, Haupt, Jänig and Kohler, 1983; Jänig and Koltzenberg, 1991). The present study demonstrates that bradykinin is also capable of mediating hyperreflexia through the prevertebral parasympathetic ganglia by activating peripheral afferent fibers which originate from myenteric plexus ganglia. Axon collaterals from primary afferent fibers are not involved, based upon the results obtained with capsaicin in Chapter 4. However, the involvement of axon collaterals from primary afferent fibers to neurons in the myenteric plexus ganglia can not be ruled out. It is possible that bradykinin acts on the same type of primary afferent terminals to induce or facilitate both peripheral and central reflexes. The axon collaterals from the primary afferents synapse with cholinergic neurons in myenteric plexus and those cholinergic neurons further synapse with neurons in the pelvic plexus ganglia.

The present study suggests that hyperreflexia in the visceral organs, induced by inflammation, is mediated through both central nervous system and pelvic plexus ganglia. It also implicates that neurons in pelvic plexus ganglia may play an important role under both physiological and pathological conditions.

The effects of human recombinant interleukin-1ß on synaptic transmission and on the active and passive properties of neurons in pelvic plexus ganglia.

The data show that hrIL-1ß causes membrane depolarization corresponding to an inward membrane current and hyperpolarization corresponding to an outward membrane current in neurons located in the guinea-pig pelvic plexus ganglia. Membrane depolarization and membrane hyperpolarization are associated with a decrease and an

increase in membrane input resistance, respectively. These effects are mediated by IL-1ß receptors, since the hrIL-1ß-evoked responses are blocked by the IL-1ß antagonist. In contrast, the actions of hrIL-1ß are not altered by blocking nicotinic, muscarinic, α_2-adrenergic or opioid receptors. Also, blockade of voltage dependent sodium and potassium channels or ω-conotoxin sensitive voltage-dependent calcium channels does not affect the hrIL-1ß-evoked responses.

The hrIL-1ß induced outward membrane current has a reversal potential of -5 ± 4.5 mV. The ionic mechanism of the hrIL-1ß-evoked outward current was not further studied in the present dissertation work. The possible mechanism for the hrIL-1ß-evoked outward current could be: 1) an increase in K^+ or Cl^- conductance; 2) activation of Na^+-K^+ pump; 3) decrease in resting Na^+ conductance and 4) decrease in Ca^{2+} conductance. Based upon the reversal potential which was obtained near -5 mV and the input resistance change associated with the hyperpolarization (increase input resistance and decrease membrane conductance), the possible involvement of potassium channels can be ruled out as the reversal potential for potassium current is near -85 mV. Furthermore, the hrIL-1ß-evoked membrane hyperpolarization or outward membrane current are associated with an increase in membrane input resistance (decrease membrane conductance), which excludes the possibility of increase in K^+ conductance. The equilibrium potential for Cl^- is -15 mV in myenteric and sympathetic ganglion neurons when 2 M KCl filled electrodes are used. At resting membrane potential (-50 - -75 mV), there will be an inward current mediated by Cl^- efflux. This resting Cl^- current may be significant in contributing to the resting membrane potential in neurons of pelvic plexus ganglia. Inhibition of this resting Cl^- current will cause membrane hyperpolarization associated with a decrease in input resistance. Thus based upon the reversal potential and the resting Cl^- current mechanism, hrIL-1ß-evoked membrane hyperpolarization or outward membrane current can be due to blocking the resting Cl^- current. However, based upon the reversal potential and membrane input resistance change, it is also reasonable to

suggest that a decrease in a cation conductance may be involved in the hrIL-1ß-evoked membrane hyperpolarization or outward membrane current. To further determine the ionic mechanism of hrIL-1ß-induced hyperpolarization, ionic substitution experiments should be performed. In Aplysia neurons, hrIL-1ß causes membrane hyperpolarization and an outward membrane current with the reversal potential near +15 mV. Ion substitution and pharmacology studies show that low $[Ca^{2+}]_o$, Ca^{2+} channel blocking cation, increasing $[K^+]_o$, TEA, 4-aminopyridine (4-AP), low $[Cl^-]_o$, ouabain and TTX have no effects on hrIL-1ß-evoked outward membrane current. Thus it is concluded that the hrIL-1ß-evoked outward membrane current is independent of a decrease in Ca^{2+} conductance, an increase in K^+ or Cl^- conductance, an increase in Ca^{2+} activated K^+ conductance and an activation of Na^+-K^+ pump. In contrast, a decrease in $[Na^+]_o$ reduced hrIL-1ß-evoked outward membrane current, indicating that in Aplysia neurons, the effect of hrIL-1ß is exclusively due to a decrease in resting Na^+ conductance. (Sawada, Hara and Maeno, 1991). A similar decrease in resting Na^+ conductance and decrease in Ca^{2+} conductance and increase in Cl^- conductance with net membrane input resistance increase may also be involved in hrIL-1ß-evoked outward membrane current or membrane hyperpolarization in neurons of pelvic plexus ganglia.

The ionic mechanism for hrIL-1ß-evoked inward current and membrane depolarization was also not further studied in the present study. However, the following mechanisms may be involved. (1) an increase Na^+ and/or Ca^{2+} conductance; 2) a decrease in K^+ conductance; 3) an increase in resting Cl^- conductance and 4) an inhibition of Na^+-K^+ pump. The hrIL-1ß-evoked membrane depolarization or intward membrane current are associated with a decrease in membrane input resistance (increase membrane conductance), therefore it is unlikely decrease in K^+ conductance is involved. However, it is possible that increase in resting Cl^- conductance and increase in cation conductance are involved in the mechanism for hrIL-1ß-induced depolarization associated with a decrease in membrane input resistance.

The actions of hrIL-1ß on the membrane potential and membrane input resistance of parasympathetic neurons in pelvic plexus ganglia are similar to the actions of hrIL-1ß found in other neurons. In rat hypothalamic supraoptic neurons, IL-1ß causes membrane depolarization and hyperpolarization and increases neuronal excitability (Li, Inenage, Kawano, Kannan and Yamashita, 1992). In hippocampal pyramidal neurons, hrIL-1ß reduces voltage dependent-calcium currents (Plata-Salamán and Ffrench-Mullen, 1992).

The effects of hrIL-1ß are similar to the actions of other inflammatory mediators. For example, platelet-activating factor causes membrane depolarization in cultured rat myenteric neurons (Willard, 1992). Recombinant human tumor necrosis factor (rhTNF) causes a slow inward current associated with a decrease in membrane conductance in Aplysia abdominal ganglion neurons (Swada, Hara and Maeno, 1990). Human recombinant interleukin-2 depressed the voltage-dependent inward calcium current in guinea-pig hippocampal CA1 neurons (Plata-Salamán and Ffrench-Mullen, 1993). In guinea-pigs actively antigen sensitized airway tissue, antigen challenge-caused membrane depolarization and change in membrane excitability in bronchial parasympathetic ganglion neurons caused by the release of inflammatory mediator(s) including IL-1ß (Myers, Undem and Weinreich, 1991).

The present results also indicate that hrIL-1ß depresses the f-EPSPs and blocks orthodromic action potentials evoked in a portion of neurons in pelvic plexus ganglia by electrical stimulation of pelvic, hypogastric and urethra-urinary bladder nerves. This suggests that there are IL-1ß receptors located presynaptically, that act to depress electrically evoked release of acetylcholine from preganglionic fibers in pelvic, hypogastric and urethra-urinary bladder nerves. The depression of the f-EPSPs occurred when the hrIL-1ß-evoked membrane depolarizations or hyperpolarizations were nullified by direct hypepolarizing or depolarizing current injection, respectively. The effects are not due to alteration of the sensitivity of the nicotinic postsynaptic receptors, since hrIL-1ß has virtually no effect on nicotinic depolarizations evoked by acetylcholine. The data

show that hrIL-1ß modulates not only the synaptic inputs from the central nervous system, but also the synaptic inputs from nerve trunks that connect peripheral organs to pelvic plexus ganglia. A presynaptic action of hrIL-1ß has been suggested in rat myenteric nerves, where hrIL-1ß suppresses the evoked release of acetylcholine or norepinephrine (Collins, Hurst, Main, Stainley, Khan, Blennerhassett and Swain, 1992; Hurst and Collins, 1993; Main, Blennerhassett and Collins, 1993). In the rat hippocampus neurons, hrIL-1ß inhibits long-term potentiation (Bellinger, 1993) and decreases the release of acetylcholine (Rada, Mark, Vitek, Mangano, Blume, Beer and Hoebel, 1991) .

In summary, the results of the present study show that IL-1ß affects the electrical properties and synaptic transmission of neurons in pelvic plexus ganglia. It is possible that during inflammation, IL-1ß and other cytokines are released from blood activated macrophages (Dinarello, 1985, 1988), macrophage-like cells (Faussone-Pellegrini, Pantalone and Cortesini, 1990; Jessen and Thuneberg, 1991), vascular smooth muscles (Warner, Auger and Libby, 1987) or neurons in pelvic plexus ganglia (Freidin, Bennett and Kessler) to directly affect sacral parasympathetic efferent pathways. Furthermore, I speculate that alteration in sacral parasympathetic reflexes during inflammation involves the action(s) of inflammatory mediator(s) on both sacral visceral afferent (Häbler, Jänig and Koltzenburg, 1990) and sacral parasympathetic efferent pathways.

Overall, the studies in this chapter show that inflammatory mediators such as bradykinin and interleukin-1ß are capable of modulating pelvic plexus ganglia-mediated peripheral reflexes at both afferent limb (bradykinin) and center station (pelvic plexus ganglia) of the reflexes (interleukin-1ß). It has been shown that both bradykinin and interleukin-1ß are released during inflammation and the release of interleukin-1ß can further induce the release of bradykinin and other hyperalgesic mediators (Ferreira, Lorenzetti, Cunha and Poole, 1993). Furthermore, both bradykinin and interleukin-1ß are involved in inducting hyperalgesia by directly acting on afferent terminals (Juan and

Lembeck, 1974; Steranka, Manning, de Haas, Ferkany, Borosky, Connor, Vavrek, Stewart, and Snyder, 1988; Ferreira, Lorenzetti, Cunha and Poole, 1993; Watkins, Wiertelak, Goehler, Smith, Martin and Maier, 1994). The studies suggest that bradykinin and interleukin-1ß are involved in the mechanisms of hyperalgesia during inflammation. It is possible that bradykinin and/or interleukin-1ß are involved in producing discomfort and pain in patients with inflammatory bowel disease (IBD).

Besides its action on afferent terminals, interleukin-1ß has been shown to inhibit cholinergic synaptic transmission in neurons of pelvic plexus ganglia from both central and peripheral nerves. Studies in the myenteric plexus have also shown that interleukin-1ß suppressed the release of acetylcholine from myenteric terminals (Collins, Hurst, Main, Stanley, Khan, Blennerhassett and Swain, 1992). These suggest that interleukin-1ß may be also involved in inhibition of inflammation-induced hyperreflexia. The protective role of interleukin-1ß in IBD has been suggested (Cominelli, Nast, Lierena, Dinarello and Zipser, 1990). Hyperalgesia and hyperreflexia are both present in patients with IBD. Bradykinin has been known to be involved in both hyperalgesia and hyperreflexia. My dissertation work has demonstrated that hyperreflexia can be mediated not only at the level of the central nervous system but also at the level of autonomic ganglia. The involvement of the pelvic plexus ganglia in mediating excitatory reflexes and the effects of bradykinin on the afferent limb of this reflex may contribute in part of the hyperreflexia in patients with IBD. Others have demonstrated hyperalgisia caused by interleukin-1ß (Ferreira, Lorenzetti, Cunha and Poole, 1993; Watkins, Wiertelak, Goehler, Smith, Martin and Maier, 1994). However, the functional role of interleukin-1ß in regulation of central and peripheral reflexes at physiological condition and in patients with IBD is uncertain and remains to be further studied.

SUMMARY

1. The passive and active electrophysiological properties of neurons in pelvic plexus ganglia, isolated from male guinea-pigs, were investigated. The neurons were not spontaneously active and had a resting membrane potential of -56.3 ± 0.3 mV (n=89); membrane input resistance of 51.4 ± 2.5 MΩ (n=82); membrane time constant of 6.5 ± 0.3 ms (n=76); and amplitude of action potentials of 73.0 ± 0.5 (n=79). The membrane properties of neurons in the pelvic plexus ganglia are similar to the properties obtained in neurons located in other parasympathetic ganglia.

2. Intracellular recordings were obtained *in vitro* from neurons in pelvic plexus ganglia of male guinea-pigs with attached central (hypogastric and pelvic) and peripheral (colonic-rectal and urethra-urinary bladder) nerves and with (n=202) or without (n=245) an attached segment of distal colon *via* colonic-rectal nerves.

3. When a segment of distal colon was not attached to the pelvic plexus ganglia, neurons in pelvic plexus ganglia received synaptic input(s) consisting of fast excitatory postsynaptic potentials (f-EPSPs) and/or action potentials during electrical stimulation of hypogastric nerve (84%, 205 of 245), pelvic nerves (80%, 102 of 128), colonic-rectal nerves (67%, 108 of 161) and urethra-urinary bladder (80%, 109 of 135) nerves. The synaptic responses were reversibly blocked by hexamethonium (C_6, 10 - 100 μM) and dihydro-ß-erythroidine (10 - 50 μM) but not by atropine (0.1 - 1.0 μM) indicating that nicotinic but not muscarinic acetylcholine receptors are involved.

4. When pelvic plexus ganglia were attached to a segment of distal colon via colonic-rectal nerves, 27% (54 of 202) of the neurons tested exhibited continuous electrical activities consisting of f-EPSPs and action potentials which were reversibly

blocked by C_6 (10 - 100 μM) applied to the ganglia compartment of the organ bath and irreversibly abolished by sectioning the colonic-rectal nerves.

5. Distension of distal colon segment increased the amplitude and frequency of f-EPSPs or the frequency of action potentials in neurons which exhibited continuous electrical activities. In quiescent neurons, distension of the distal colon initiated f-EPSPs and/or action potentials. The results suggest that neurons in pelvic plexus ganglia receive sensory inputs from mechanoreceptors located in the distal colon wall. These sensory inputs involve nicotinic acetylcholine receptors.

6. Superfusion of the distal colon segment with a Krebs solution containing bradykinin (1 nM) initiated f-EPSPs and action potentials in quiescent neurons of pelvic plexus ganglia. The frequency and the amplitude of f-EPSPs was further increased by colon distension. The data suggest that bradykinin sensitizes the mechanosensory input from distal colon-rectum to neurons in pelvic plexus ganglia.

7. Reflexes to colon and urinary bladder in male guinea-pigs were studied *in vitro* using preparations with pelvic plexus ganglia attached to two separated segments of distal colon via colonic nerves or to distal colon and urethra-urinary bladder via colonic nerves and urethra-urinary bladder nerves. Passive distension of one colon segment or electrical stimulation of colonic nerves (3-20 Hz) evoked contractile responses of the other colonic segment and urinary bladder. Electrical stimulation of urethra-urinary bladder nerves (3-20 Hz) also evoked colon contractile responses. The data suggest that neurons in pelvic plexus ganglia mediate excitatory reflexes to colon and urinary bladder.

8. The contractile response to passive distension of one of the colon segments or to electrical stimulation of colonic or urethra-urinary bladder nerves were blocked by tetrodotoxin (TTX, 1 μM), hexametonium (C_6, 10 μM) and atropine (1 μM). Also, these responses were abolished by sectioning the colonic or urethra-urinary bladder nerves. The data indicate that reflex contractions involve cholinergic nerve fibers which go with

the colonic and urethra-urinary bladder nerves and activate nicotinic and muscarinic acetylcholine receptors.

9. The action of human recombinant interleukin-1ß (hrIL-1ß) was tested on neurons in isolated guinea-pig pelvic plexus ganglia, utilizing *in vitro* intracellular microelectrode recording techniques. Pressure or bath application of hrIL-1ß caused membrane depolarization (3-32 mV) and an inward current (0.5-1.0 nA) in 54% of neurons tested. The depolarization was associated with a decrease in membrane input resistance. HrIL-1ß also caused membrane hyperpolarization (3-14 mV) and an outward current (0.3-0.8 nA) in 30% of neurons tested. The hyperpolarization was associated with an increase in membrane input resistance.

10. Membrane depolarization, hyperpolarization and associated changes in membrane input resistance evoked by hrIL-1ß were resistant to hexamethonium (100 µM), atropine (500 nM), yohimbine (300 nM) or naloxone (1 µM) as well as to tetrodotoxin (5 µM), ω-conotoxin (700 nM) and tetraethylammonium (30 mM). The effects of hrIL-1ß on membrane potential and membrane input resistance were blocked by the IL-1ß receptor antagonist (10-40 µg/ml). The data indicate that the effects of hrIL-1ß on the membrane potential and input resistance of neurons in pelvic plexus ganglia are mediated by specific IL-1 receptors and do not involve activation of nicotinic and muscarinic acetylcholine receptors, α_2-adrenergic and opioid receptors as well as changes in the transmembrane currents carried through voltage-dependent Na^+, Ca^{2+} or K^+ channels.

11. Electrical stimulation of pelvic, hypogastric or urethra-urinary bladder nerves evoked f-EPSPs and action potentials, which were reversibly blocked by hexamethonium (10-100 µM) or dihydro-ß-erythroidine (5-100 µM), indicating that the responses are due to activation of cholinergic nicotinic receptors. In 44% of neurons tested (n=7), hrIL-1ß inhibited the orthodromic action potentials and f-EPSPs in a dose-dependent fashion. The effects of hrIL-1ß on synaptic transmission were blocked by the IL-1ß receptor

antagonist (40 μg/ml). HrIL-1ß had no effects on the depolarizations due to activation of nicotinic receptors by pressure application of acetylcholine. The data suggest that hrIL-1ß does not alter the sensitivity postsynaptic nicotinic acetylcholine receptors. Thus, the inhibition of cholinergic synaptic transmission in pelvic plexus ganglia by hrIL-1ß is most probably due to activation of IL-1ß receptors on presynaptic nerve terminals, which inhibit the release of acetylcholine.

In summary, the data obtained in the present studies show that neurons in pelvic plexus ganglia receive mechanoreceptor-mediated synaptic inputs from distal colon. These neurons are capable of mediating reflexes between colon segments and between the colon and the urinary bladder. The data also suggest that inflammatory mediators, such as bradykinin and interleukin-1β, may regulate these peripheral reflexes at both, the afferent site and at the level of the pelvic plexus ganglia. The pelvic plexus ganglia-mediated visceral organ reflexes may play a significant role in modulating the defecation and micturition reflexes under both physiological and pathological conditions.

SIGNIFICANCE OF THE STUDY

Classically, afferent information from distal colon, the rectum and anal canal is received in the sacral and lumbar regions of the spinal cord via the pelvic and pudendal nerve and via the hypogastric and inferior lumbar splanchnic nerves. These afferent fibers are involved in the initiation of spinal autonomic and somatic reflex mechanisms and the transmission of visceral and somatic sensation to higher centers in the brain. Afferent information from distal colon-rectum is also received by the intramural plexus ganglion neurons and involved in local gastrointestinal reflexes. Both types of reflexes participate in defecation. The present study established a novel reflex pathway which may also be involved in autonomic and defecation reflexes. The afferents of this pathway send information to neurons in pelvic plexus ganglia, a parasympathetic ganglia. Most pelvic plexus ganglia efferents innervate visceral organs of the pelvic region, and mediate colon-colonic reflex and colon-urinary bladder reflex. These pathways may participate in the defecation reflex, micturition reflex and regulate distal colon and urinary bladder motilities under either physiological (normal defecation) and pathological conditions (eg. defecation under spinal cord injury condition). Since the present studies also demonstrated that inflammatory mediators are able to modulate the afferent information to neurons in pelvic plexus ganglia and alter pelvic plexus ganglia-mediated reflexes, this reflex pathway may play a significant role in patients with inflammatory visceral organ diseases.

The present dissertation work have been published in abstract form (Lin and Krier, 1993a,b, 1994, 1995) and the work in Chapter 1, 2, 3, and 4 are in preparation for publication as a full manuscript. The dissertation work in Chapter 5 has been accepted as a full manuscript and will be published in *American Journal of Physiology*, 1995.

BIBLIOGRAPHY

BIBLIOGRAPHY

Aarden, L. A., T. K. Brunner, J. C. Cerottini (1979) Revised nomenclature for antigen-non-specific T-cell proliferation and helper factors. *J. Immunol.* 123: 2928-2929 *(letter)*.

Adamson, W. A. D. and I. Aird (1932) Megacolon: evidence in favor of a neurogenic origin. *Br. J. Surg.* 20: 220-233.

Amara, S. G., V. Jonas, M. G. Rosenfeld, E. S. Ong and R. M. Evans (1982) Alterative RNA processing in calcitonin gene expression generates mRNA encoding different polypeptide products. *Nature.* 298:240-244.

Baker, D. G., H. M. Coleridge, J. C. Coleridge and T. Nerdrum (1980) Search for cardiac nociceptor: stimulation by bradykinin of sympathetic afferent nerve endings in the heart of the cat. *J. Physiol. Lond.* 306: 519-536.

Bahns, E., U. Ernsberger, W. Jänig and A. Nelke (1986) Functional characteristics of lumbar visceral afferent fibers from the urinary bladder and urethra in the cat. *Pflüg. Arch.* 407: 510-518.

Bauer, A. J., M. Hanani, T. C. Muir and J. H. Szurszewski (1991) Intracellular recordings from gallbladder ganglia of opossums. *Am. J. Physiol.* 260:G299-G306.

Bellinger, F. P., S. Madamba and G. R. Siggins (1993) Interleukin 1ß inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. *Brain Res.* 628: 227-234.

Billingham, M. E. J. (1987) cytokine as inflammatory mediators. *British Med. Bull.* 43(2): 350-370.

Bernheim, H. A., L. H. Block and E. Atkins (1979) Fever: pathogenesis, pathophysiology, and purpose. *Ann. Intern. Med.* 91: 261-270.

Bevan, S. J. and C. A. Forbes (1988) Membrane effects of capsaicin on rat dorsal root ganglion neurons in cell culture. *J. Physiol. (Lond). 398: 28P.*

Blackman, J. G., J. P. Crowcroft, C. E. Devine, M. E. Holman and K. Yonemura (1969) Transmission from preganglionic fiberes in the hypogastric nerve to peripheral ganglia of male guinea- pigs. *J. Physiol.* 201:723-743.

Breder, C. D., C. A. Dinarello and C. B. Saper (1988) Interleukin-1 immunoreactivation of the human hypothalamus. *Science*. 240(4850): 321-324.

Brugger, F. , R. H. Evans and N. S. Hawkins (1990) Effects of N-methyl-Daspartate antagonists and spantide on spinal reflexes and responses to substance P and capsaicin in isolated spinal cord preparations from mouse and rat. *Neuroscience* 36:611-622.

Buck, S. H. and E. Burcher (1986) The tachykinins: a family of peptides with a brood of receptors. *Trends Pharmacol. Sci.*. 7: 65-68.

Buck, S. H. and T. F. Burks (1986) The neuropharmacology of capsaicin: a review of some recent observations. *Pharmacol. Rev.* 38:179-226.

Buck, S. H., R. M. Pruss, J. L. Krstenansky, P. J. Robinson and K. A. Stauderman (1988) A tachykinin peptide receptor joins an elite club. *Trends Pharmacol. Sci.*. 9: 3-5.

Burgess, G. M., I. Mullaney, M. McNeill, P. R. Coote, A. Minhas and J. N. Wood (1989) Activation of guanylate cyclase by bradykinin in rat sensory neurons is mediated by calcium influx: possible role of the increase in cyclic GMP. *J. Neurochem.* 53(4): 1212-1218.

Burgess, G. M., I. Mullaney, M. McNeill, P. M. Dunn and H. P. Rang (1989) Second messengers involved in the mecdhanism of action of bradykinin in sensory neurons in culture. *J. Neurosci.* 9(9): 3314-3325.

Caranikas, S., J. Mizrahi, P. D'Orléans-Juste and D. Regoli (1982) Antagonist of substance P. *Eur. J. Pharmacol.* 77: 205-206.

Cevero, F. and K. A. Sharkey (1988) An electrophysiological and anatomical study of intestinal afferent fibers in the rat. *J. Physiol. Lond.* 401:381-397.

Chahl, L. A. (1982) Evidence that the contractile response of guinea-pig ileum to capsaicin is due to substance P release. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 319: 212-215.

Chahl, L. A. and A. Iggo (1977) The effects of bradykinin and PGE_2 on rat cutaneous afferent nerve activity. *Br. J. Pharmacol.* 59:343-347.

Collins, S. M., S. M. Hurst, C. Main, E. Stanley, I. Khan, P. Blennerhassett and M. Swain (1992) Effect of inflammation on enteric nerves: cytokine-induced changes in neurotransmitter content and release. *Ann. N.Y. Acad. Sci.* 664: 415-424.

Cominelli, F., C. C. Nast, B. D. Clark, R. Schindler, R. Lierena, V. E. Eysselein, R. C. Thompson and C. A. Dinarello (1990) Interleukin 1 (IL-1) gene expression,

synthesis, and effect of specific IL-1 receptor blockade in Rabbit immune complex colitis. *J. Clin. Invest.* 86:972-980.

Cominelli, F., C. C. Nast, R. Lierena, C. A. Dinarello and R. D. Zipser (1990) Interleukin 1 suppresses inflammation in rabit colitis mediation by endogenous prostaglandins. *J. Clin. Invest.* 85:582-586.

Cooke, A. R. (1975) Control of gastric emptying and motility. *Gastroenterology* 68:804-816.

Costa, M. and J. B. Furness (1976) The peristaltic reflex: an analysis of nerve pathways and their pharmacology. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 294:47-60.

Cottrell, D. F. and A. Iggo (1984) The responses of duodenal tension receptors in sheep to pentagastrin, cholecystokinin and other drugs. *J. Physiol. Lond.* 354: 477-495.

Crowcroft, P. J., M. E. Holman and J. H. Szurszewski (1971) Exitatory input from the distal colon to the inferior mesenteric ganglion in the guinea-pig. *J. Physiol.* 219:443-461.

Crowcroft, P. J. and J. H. Szurszewski (1971) A study of the inferior mesenteric and pelvic ganglia of guinea-pigs with intracellular electrodes. *J. Physiol.* 219: 421-441.

Dalsgaard, C.-J., M. Vincent, T. Schultzberg, T. Hökfelt, L.-G. Elfvin, L. Terenius and G. J. Dockray (1983) Capsaicin-induced depletion of substance P-like immunoreactivity in guinea pig sympathetic ganglia. *J. Autonomic Nervous System.* 9: 595-606.

Daniel, E. E., M. B. Parrish, E. G. Watson, J. E. T. Fox-Threlkeld, D. Regoll and K. D. Rainsford (1995) The tachykinin receptors including contractile responses of canine ileum circular muscle. *Am. J. Physiol.* 268 (*Gastrointest. Liver Physiol.* 31): G161-G170.

Davey, N. J. and P. H. Ellaway (1989) Facilitation of individual γ-motoneurones by the discharge of single slowly adapting type 1 mechanoreceptors in cats. *J. Physiol.* 411:97-114.

Davies, P. J., H. A. Coleman and H. C. Parkington (1993) Phasic and tonic firing neurons in the pelvic plexus of the female guinea-pig. *XXXII Congress of the International Union of Physiological sciences* 96.1/P, 84.

Dayer, J. M., D. R. Robinson and S. M. Krane (1977) Prostaglandin production by rheumatoid synovial cells: stimulation by a factor from human mononuclear cells. *J. Exp. Med.* 145: 1399-1404.

Decktor, D. L. and W. A. Weems (1981) A study of renal-efferent neurons and their neural connexions within cat renal ganglia using intracellular electrodes. *J. Physiol.* 321:611-626.

Decktor, D. L. and W. A. Weems (1983) An intracellular characterization of neurons and neural connexions within the left coeliac ganglion of cats. *J. Physiol.* 341:197-211.

de Groat, W. C. and J. Krier (1975) Preganglionic C-fibers: A major component of the sacral autonomic outflow to the colon of the cat. *Pflügers Arch.* 359:171-176.

de Groat, W. C. and J. Krier (1976) An electrophysiological study of the sacral parasympathetic pathway to the colon of the cat. *J. Physiol. Lond.* 260: 425-445.

de Groat, W. C. and J. Krier (1978) The sacral parasympathetic reflex pathway regulating colonic motility and defecation in cat. *J. Physiol. Lond.* 276: 481-500.

de Groat, W. C., I. Nadelhaft, R. J. Milne, A. M. Booth, C. Morgan and C. K. Thor (1981) Organization of the sacral parasympathetic reflex pathways to the urinary bladder and large intestine. *J. Autonomic Nervous System.* 3: 135-160.

Dinarello, C. A. (1985) An update on human interleukin-1: from molecular biology to clinical relevance. *J. Clin Immunol.* 5: 287-297.

Dinarello, C. A (1988) Biology of interleukin 1. *FASEB* 2(2): 108-115.

Dinarello, C. A and R. C. Thompson (1991) Blocking IL-1: interleukin 1 receptor antagonist in vivo and in vitro. *Immunol. Today.* 12(11): 404-410.

Dingle J. T., J. Saklatvala, R. Hembry, J. Tyler, H. V. Fell and R. A. Jubb (1979) A cartilage catabolic factor from synovium. *Biochem. J.* 184: 177-180.

Docherty, R. J., B. Robertson ans S. Bevan (1991) Capsaicin causes prolonged inhibition of voltage-activated calcium currents in adult rat dorsal root ganglion neurons in culture. *Neuroscience.* 40 (2): 513-521.

Dripps, D. J., E. Verderber, R. K. Ng, R. C. Thompson and S. P. Eisenberg (1991) Interleukin-1 receptor antagonist binds to the type II interleukin-1 receptor on B cells and neutrophils. *J. Biol. Chem.* 266(30): 20311-20315.

Dun, N. J. and S. Minota (1981) Effects of substance P on neurones of the inferior mesenteric ganglia of the guinea-pig. *J. Physiol.* 321:259-271.

Dunn, P. M. and H. P. Rang (1990) Bradykinin-induced depolarization of primary afferent nerve terminal in the neonatal rat spinal cord *in vitro*. *Br. J. Pharmacol.* 100: 656-660.

Eastgate, J. A., J. A. Symons and G. W. Duff (1990) Identification of an interleukin-1 beta binding protein in human plasma. *FEBS Lett.* 260(2): 213-216.

Eisenberg, S. P., R. J. Evans, W. P. Arend, E. Verderber, M. T. Brewer, C. H. Hannum and R. C. Thompson (1990) Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. *Nature.* 343(6256): 341-346.

Erspamer, V. (1981) The tachykinin peptide family. *Trends Neurosci.* 4:267-269.

Faussone-Pellegrini, M. S. D. Pantalone and C. Cortesini (1990) Smooth muscle cells, interstitial cells of Cajal and myenteric plexus interrelationships in the human colon. *Acta. Anat. Basel.* 139: 31-34.

Ferreira, S. H., B. B. Lorenzetti, F. Q. Cunha and S. Poole (1993) Bradykinin release of TNF-α plays a key role in the development of inflammatory hyperalgesia. *Agents Actions. Special Conference Issue.* 38: C7-C9.

Floyd, K., V. E. Hick and J. F. B. Morrison (1976) Mechanosensitive afferent units in the hypogastric nerve of the cat. *J. Physiol.* 259:457-471.

Floyd, K., V. E. Hick, J. Koley and J. F. B. Morrison (1977) The effects of bradykinin on afferent units in intra-abdominal sympathetic nerve trunks. *Q. J. Exp. Physiol.* 62: 19-25.

Freidin, M., M. V. Bennett and J. A. Kessler (1992) Cultured sympathetic neurons synthesize and release the cytokine interleukin 1 beta. *Proc. Natl. Acad. Sci. USA.* 89 (21): 10440-10443.

Garry, R. C. (1933) The nervous control of the caudal region of the large bowel in the cat. *J. Physiol. London.* 77:422-431.

Garry, R. C. (1934) The movement of the large intestine. *Physiol. Rev.* 14:103-132.

Gallagher, J. P. and P. Shinnick-Gallagher. (1986) Excitatory transmission in parasympathetic ganglia. In: *Autonomic and Enteric Ganglia.* New York: Plenum: 341-351.

Gammon C. M., A. C. Allen and P. Morell (1989) Bradykinin stimulates phosphoinositide hydrolysis and mobilization of arachidonic acid in dorsal root ganglion neurons. *J. neurochem.* 53(1): 95-101.

Gauldie, J. (1991) Acute and chronic inflammation. *In Effects of immune cells and inflammation on smooth muscle and enteric nerves.* W. J. Snape and S. M. Collins Eds. 1-10. CRC Press, Inc. Boca Raton.

Gery, I. and B. H. Waksman (1972) Potentiation of the T-lymphocyte response to mitogens 11. The cellular source of potentiating mediator(s). *J. Exp. Med.* 136: 143-155.

Gibbins, I. L., J. B. Furness and M. Costa (1987) Pathway-specific patterns of the co-existence of substance P, calcitonin gene-related peptide, cholecystokinin and dynorphin in neurons of the dorsal root ganglia of the guinea-pig. *Cell Tissue Res.* 248: 417-437.

Gibbins, I. L., J. B. Furness and M. Costa, I. MacIntyre, C. J. Hillyard and S. Girgis (1985) Co-localization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs. *Neurosci Lett.* 57: 125-130.

Gillespie, J. S. and J. D. Maxwell (1971) Adrenergic innervation of the sphincteric and non-sphincteric smooth muscle in the rat intestine. *J. Histochem. Cytochem.* 19: 676-681.

Giulian, D., R. L. Allen, T. J. Baker and Y. Tomozawa (1986) Brain peptides and glial growth I. Glia-promoting factors as regulators of gliogenesis in the developing and injured central nervous system. *J. Cell. Biol.* 102(3): 803-811.

Goodman, E. C. and L. L. Iversen (1986) Calcitonin gene-related peptide: novel neuropeptide. *Life Sci.* 38: 2169-2178.

Goyal, R. K., J. N. Sengupta and J. K. Saha (1992) Properties of esophageal mechanosensitive receptors. *Advances in the Innervation of the Gastrointestinal Tract. G. E. Holle et al. editors.* 523-537.

Granowitz, E. V., B. D. Clark, J. Mancilla and C. A. Dinarello (1991) Interleukin-1 receptor antagonist competitively inhibits the binding of interleukin-1 to the type II interleukin-1 receptor. *J. Biol. Chem.* 266(22): 14147-14150.

Griesbacher, T. and F. Lembeck (1987) Actions of bradykinin antagonists on bradykinin-induced plasma extravasation, prostaglandin E_2 release, nociceptor stimulation and contraction of the iris sphincter muscle of the rabbit. *Br. J. Pharmacol.* 92: 333-340.

Griffith III,W. H., J. P. Gallagher and P. Shinnick-Gallagher (1980) An intracellular investigation of cat vesical pelvic ganglia. *J. Neurophysiol.* 43:343-354.

Grundy, D. (1985) *Gastrointestinal Motility. The Integration of Physiological Mechanisms.* Lancaster, UK: MTP.

Grundy, D. and T. Scratcherd (1989) Sensory afferents from the gastrointestinal tract. *Handbook of Physiology. The Gastrointestinal system I.* 593-620.

Guilbaud G., D. Lebars and J. M. Besson (1976) Bradykinin as a tool in neurophysiological studies of pain mechanisms. In: *Advances in pain research and therapy*, edited by J. J. Bonica and D. Albe-Fassard. New York: Raven. p. 67-73.

Guzman, F., D. Braun and R. K. S. Lim (1962) Visceral pain and pseudoaffective response to intra-arterial injection of bradykinin and other algesic agents. *Arch. Int. Pharmacodyn. Ther.* 136: 353-384.

Häbler, H. J., W. Jänig and M. Koltzenburg (1988) A novel type of unmyelinated chemosensitive nociceptor in the acutely inflamed urinary bladder. *Agents Actions.* 25: 219-221.

Häbler, H. J., W. Jänig and M. Koltzenburg (1989) Activation of chemosesitive C-fibers by an acute inflammation of the urinary bladder of the cat. *J. Physiol. (Lond.).* 415: 65.

Häbler, H. J., W. Jänig and M. Koltzenburg (1990) Activation of Unmyelinated afferent fibers by mechanical stimuli and inflammation of the urinary bladder in the cat. *J. Physiol. Lond.* 425: 545-562.

Häbler, H. J., W. Jänig, M. Koltzenburg and S. B. McMahon (1990) A quantitative study of the central projection patterns of unmyenlinated ventral root afferents in the cat. *J. Physiol.* 422:265-287.

Hall, A. K., P. J. Barnes, L. A. Meldrum and H. J. Maclagau. (1989) Facilitation by tachykinins of neurotransmission in guinea-pig pulmonary parasympathetic nerves. *Br. J. Pharmacol.* 97:274-280.

Handwerker, H. O. (1976) Influence of algogenic substances abd prostaglandins on the discharges of unmyenlinated cutaneous nerve fibers identified as nociceptors. In: *Pain Research and Therapy,* edited by J. J. Bonic and D. Albe-Fessard. New York: Raven, vol. 1, p. 41-45.

Handwerker, H. O. (1980) Pain producing substances. In: *Pain and Society* edited by H. W. Kosterlitz and L. Y. Terenius. Weinheim, F.R.G: VCH p. 325-338.

Hannum, C. H., C. J. Wilcox, W. P. Arend, F. G. Joslin, D. J. Dripps, P. L. Heimdal, L. G. Armes, A. Sommer, S. P. Eisenberg and R. C. Thompson (1990) Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature.* 343: 336-340.

Hardy, J. D., H. G. Wolff and H. Goodell (1950) Experimental evidence on the nature of cutaneous hyperalgesia. *J. Clinic. Invest.* 29:115-140.

Hartman, D. and J. Krier (1984) Synaptic and antidromic potentials of visceral neurons in ganglia of the lumbar sympathetic chain of the cat. *J. physiol.* 350:423-428.

Haupt, P., W. Jänig and W. Kohler (1983) Response pattern of visceral afferent fibers, supplying the colon, upon chemical and mechanical stimuli. *Pflügers Arch.* 398:41-47.

Hertog, A. D., A. Nelemans and J. V. den Akker (1988) The multiple action of bradykinin on smooth muscle of guinea-pig taenia caeci. *Eur. J. Pharmacol.* 151: 357-363.

Heyman, I. and H. P. Rang (1985) Depolarizing responses to capsaicin in a subpopulation of rat dorsal root ganglion cells. *Neurosci lett.* 56:69-75.

Holtzer, P. (1988) Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-relared peptide and other neuropeptides. *Neuroscience.* 24 (3): 739-768.

Holtzer, P. (1991) Capsaicin: cellular targets, mechanism of action, and selectivity for the sensory neurons. *Pharmacological Reviews.* 43 (2): 143-196.

Hoyes, A. D., and P. Barber (1981) Degenerawtion of axons in the ureteric and duodenal nerve plexuses of the adult rat following in vivo treatment with capsaicin. *Neurosci. Lett. 25: 19-24.*

Hurst, S. and S. M. Collins (1993) Interleukin-1ß modulation of norepinephrine release from ratmyenteric nerves. *Am. J. Physiol.* 264 (*Gastrointes. Liver Physiol.* 27): G30-G35.

Iggo, A. (1957) Gastrointestinal tension receptors with unmyelinated fibers in the vagus of the cat. *J. Exp. Physiol.* 42:130-143.

Jacobowitz, D. (1965) Histochemical studies of the autonomic innervation of the gut. *J. Pharmacol. and Exp. Ther.* 149:358-364.

Jancsó, N. (1960) Role of the nerve terminals in the mechanism of inflammatory reactions. *Bull Millard Fillmore Hosp.* 7:53-77.

Jancsó, N. (1968) Desensitization with capsaicin and related acylamides as a tool for studying the function of pain receptors. In: *Pharmacology of Pain-Proceedings of Third International Congress on Pharmacology,* ed. by R. K. S. Lim, p. 33-55, Pergamon Press.

Jancsó, N. and C. A. Maggi (1987) Distribution of capsiacin-sensitive urinary bladder afferents in the rat spinal cord. *Brain Res.* 418:371-376.

Jancsó, G., S. Karcsu, E. Király, A. Szebeni, L. Toth, E. Bácsy, F. Joó and Á. Párducz (1984) Neurotoxin induced nerve cell degeneration: possible involvement of calcium. *Brain Res.* 295: 211-216.

Jänig, W. and M. Koltzenburg (1989) The neural basis of consciously perceived sensations from the gut. In M. V. Singer and H. Goebell (Eds.). *Nerves and the Gastrointestinal Tract.* MTP Press, Lancaster: 183-197.

Jänig, W. and M. Koltzenburg (1991) Receptive properties of sacral primary afferent neurons supplying the colon. *J. Neurophysiol.* 65:1067-1077.

Jänig, W. and J. F. B. Morrison (1986) Functional properties of spinal visceral afferents supplying abdominal and pelvic organs, with special emphasis on visceral nociception. *Progress in Brain Research. Elsevier Science Publishers B. V. (Biomedical Division)* 67:87-114.

Jessen, H. and L. Thuneberg (1991) Interstitial cells of Cajal and Auerbachs plexus. A scanning electron microscopical study of guinea-pig small intestine. *J. Submicrosc. Cytol. Pathol.* 23: 195-212.

Jordan, F. L. and W. E. Thomas (1988) Brain macrophages: questions of origin and interrelationship. *Brain Res.* 472(2): 165-178.

Juan, H. and F. Lembeck (1974) Action of peptides and other algesic agents on paravascular pain receptors of the isolated perfused rabbit ear. *Naunyn-Schmiedebergs Arch. Pharmacol.* 283: 151-164.

Julé, Y. and J. H. Szurszewski (1983) Electrophysiology of neurones of the inferior mesenteric ganglion of the cat. *J. Physiol.* 344:277-292.

Kaczmarek, L. K. (1987) The role of protein kinase C in the regulation of ion channels and neurotransmitter release. *Trends Neurosci.* 10: 30-34.

Kampschmidt, R. F. (1981) Leucocyte endogenous mediator/endogenous pyrogen. In: Powanda M. C., Canonico, P. G., eds. *The physiologic and metabolic responses of the host.* Amsterdam: Elsevier/North-Holland: 55-74.

Katori, M., Y. Hori, K. Uchida, Tanaka and Y. Harada (1986) Different modes of interaction of bradykinin with prostaglandings in pain and acute inflammation. *Adv. Exp. Med. Biol.* 198 (B): 393-398.

Kaufman, M. P., D. G. Baker, H. M. Coleridge and J. C. Coleridge (1980a) Stimulation by bradykinin of afferent vagal C-fibers with chemosensitive endings in the heart and aorta of dog. *Cir. Res.* 46: 476-484.

Kaufman, M. P., J. C. Coleridge, H. M. Coleridge and D. G. Baker (1980b) Bradykinin stimulates afferent vagal C-fibers in the intrapulmonary airways of dogs. *J. Appl. Physiol.* 48: 511-517.

Keele, C. A. and D. Armstrong (1964) *Substance P producing pain and itch.* London: Arnold, 1964.

Kennedy, C. and J. Krier (1987) δ-opiod receptors mediate inhibition of fast excitatory postsynaptic potetnials in cat parasympathetic colonic ganglia. *Br. J. Pharmac.* 92: 437-443.

King, B. F. and J. H. Szurszewski (1984) Mechanoreceptor pathways from the distal colon to the autonomic nervous system in the guinea-pig. *J. Physiol. Lond.* 350:93-108.

Kirsner, J. B. and R. G. Shorter (1988) *Inflammatory bowel disease.* 3rd edit. Lea and Febiger. Philadelphia.

Koch, T. R., A. Sonnenberg and J. A. Carney (1991) Gut neuropeptides and the pathophysiology of inflammatory bowel disease. *In Effects of immune cells and inflammation on smooth muscle and enteric nerves.* W. J. Snape and S. M. Collins, Eds. 169-180 CRC Press, Inc. Boca Raton.

Kreulen, D. L. , T. C. Muir and J. H. Szurszewski (1983) Peripheral sympathetic pathways to gastroduodenal region of the guinea pig. *Am. J. Physiol.* 245 (Gastrointest. Liver Physiol. 8): G369-G375.

Kreulen, D. L. and Peters, S. (1986) Non-cholinergic transmission in a sympathetic ganglion of the guinea-pig elicited by colon distension. *J. Physiol.* 374, 315-334.

Kreulen, D. L. and J. H. Szurszewski (1979a) Reflex pathways in the abdominal prevertebral ganglia: evidence for a colo-colonic inhibitory reflex. *J. Physiol.* 295:21-32.

Kreulen, D. L. and J. H. Szurszewski (1979b) Nerve pathways in celiac plexus of the guinea pig. *Am. J. Physiol.* 237(1):E90-E97.

Krier, J. and D. A. Hartman (1984) Electrical properties and synaptic connections to neurons in parasympathetic colonic ganglia of the cat. *Am. J. Physiol.* 247:G52-G61.

Krier, J. and J. H. Szurszewski (1982) Effect of substance P on colonic mechanoreceptors motility and sympathetic neurons. *Am. J. Physiol.* 243: G259-G267.

Kumazawa, T. and K. Mizumura (1980) Chemical responses of polymodal receptors of the scrotal contents of dogs. *J. Physiol. Lond.* 299:219-231.

Kuntz, A. and G. Saccomanno (1944) Reflex inhibition of intestinal motility mediated through decentralized prevertebral ganglia. *J. Neurophysiol.* 7: 163-170.

Kuriyama, K., T. Hori, T. Mori and T. Nakashima (1990) Action of interferon a and interleukin-1ß on the glucose-responsive neurons in the ventromedial hypothalamus. *Brain res. Bull.* 24: 803-810.

Langley, J. N. (1921) *The Autonomic Nervous System*, Part I, Heffer publications, Cambridge.

Langley, J. N. and H. K. Anderson (1896a) The innervation of the pelvic and adjoining visera. Part I. The lower portion of the intestine. *J. Physiol. Lond.* 18:67-105.

Langley, J. N. and H. K. Anderson (1896b) The innervation of the pelvic and adjoining visera. Part II. The bladder. *J. Physiol. Lond.* 19:71-84.

Langley, J. N. and H. K. Anderson (1896c) The innervation of the pelvic and adjoining visera. Part V. Position of the nerve cells on the course of the efferent nerve fibers. *J. Physiol. Lond.* 19:131-139.

Lawson, H. (1934) The role of the inferior mesenteric ganglia in the diphasic response of the colon to sympathetic stimuli. *Am. J. Physiol.* 109: 257-273.

Lawson, H. and J. P. Holt (1937) The control of the large intestine by the decentralized inferior mesenteric ganglia. *Am. J. Physiol.* 118: 780-785.

Lee, Y., K. Takami, Y. Kawai, S. Girdis, C. J. Hillyard, I. Macintyre, P. C. Emson and M. Tohyama (1985) Distribution of the calcitonin gene-related peptide in the rat peripheral nervous system with reference to its coexistence with substance P. *Neuroscience.* 15:1227-1237.

Li, Z., K. Inenage, S. Kawano, H. Kannan and H. Yamashita (1992) Interleukin-1ß directly excites hypothalamic supraoptic neurons in rats *in vitro*. *Neuroreport.* 3: 91-93.

Lichtman, J. W. (1977) The reorganization of synaptic connections in the rat submandibular ganglion during post-natal development. *J. Physiol. London.* 273: 155-177.

Ligumsky, L., P. L. Simon, F. Karmeli and D. Rachemilewitz (1990) Role of interleukin-1 in inflammatory bowel disease-enhanced production during active disease. *Gut.* 31: 686-689.

Lim, R. K. S., C. N. Liu, F. Guzman and F. Braun (1962) Visceral receptors concerned in visceral pain and psudoaffective response to intraarterial injection of bradykinin and other algesic agents. *J. Comp. Neurol.* 118: 269-293.

Lin, J. and J. Krier (1993a) Excitatory inputs from distal colon/rectum to neurons in pelvic plexus ganglia of male guinea-pig. *Abstracts, Society for Neuroscience.* 19: 1523.

Lin, J. and J. Krier (1993b) Central and peripheral synaptic inputs to neurons in guinea-pig pelvic plexus ganglia. *J. Gastrointestinal Motility.* 5 (3):200.

Lin, J. and J. Krier (1994) Peripheral excitatory reflexes to colon and urinary bladder involve mechanosensitive neurons in male guinea-pig pelvic plexus ganglia. *Abstract, Society for Neurosciences.* 20: 112, .

Lin, J. and J. Krier (1995) Human recombinant interleukin-1ß inhibits nicotinic transmission in neurons of pelvic plexus ganglia of male guinea-pigs. *Abstract, Submitted to Gastroenterology.*

Maggi, C. A. (1990) Tachykinin receptors in the airways and lung: what should we block? *Pharmacol. Res.* 22 (5): 527-540.

Main, C., P. Blennerhassett and S. M. Collins (1993) Human recombinant interleukin 1ß suppresses acetylcholine release from rat myenteric plexus. *Gastroenterology.* 104: 1648-1654.

Majno, G. (1985) Inflammatory mediators: Where are they going? IN; Higgs G. A., Williams, T. H. eds. *Infalmmatory mediators.* Basingstoke: MacMillan: 1-6.

March S. J., C. E. Stansfeeld, D. A. Brown, R. Davey, and D. McCarthy (1987) The mechanism of action of capaicin on sensory C-type neurons and their axons *in vitro. Neoroscience* 23: 275-290.

Martin, H. A., A. T. Basbaum, G. C. Kwiat, E. J. Goetzl and J. D. Levine (1987) leukotriene and prostaglandin sensitization of cutaneous high threshold C- and A-delta mechanonociceptors in the hairy skin of rat hindlimbs. *neuroscience* 22: 651-659.

Martin, A. R. and G. Pilar (1963) Transmission through the ciliary ganglion of the chick. *J. Physiol. London.* 168: 464-475.

Marzuk, P. (1985) Biofeedback for gastrointestinal disorders: a review of the literature. *Ann. Intern. Med.* 103: 240-244.

McCarthy, D. O., M. J. Kluger and A. J. Vander (1986) Effect of centrally administered interleukin-1 and endotoxin on food intake of fasted rats. *Physiol. Behav.* 36: 745-749.

McGehee, D. S., M. F. Goy and G. S. Oxford (1992) Involvement of the nitric oxide-cyclic GMP pathway in the desensitization of bradykinin responses of cultured rat sensory neurons. *Neuron.* 9(2): 315-324.

McLachlan, E. M. (1977) Interaction between sympathetic and parasympathetic pathways in the pelvic plexus of the guinea pig. *Proceedings of The Australian Physiological and Pharmacological Society.* 8: 15P.

McLellan, A. M. and H. Goodell (1943) Pain from the bladder, ureter, and kidney pelvic. *Research publications of the association of nervous and mental disorders.* 23: 252-262.

McRorie, J., J. Krier and T. Adams (1991) Morphology and projections of myenteric neurons to colonic fiber bundles of the cat. *J. Autonomic Nervous system.* 32: 205-216.

Mense, S. (1977) Nervous control of skeletal muscle following chemical noxious stimulation. *J. Physiol. Lond.* 267: 75-88.

Miller, R. J. (1987) Bradykinin highlights the role of phospholipid metabolism in the control of nerve excitability. *Trends Neurosci.* 10: 226-228.

Mizel, S. B. (1989) The interleukins. *FASEB J.* 3(12): 2379-2388.

Mizumura, K., J. Sato and T. Kumazawa (1987) Effects of prostaglandins and other putative chemical intermediaries on the activity of canine testicular polymodal receptors studied in vitro. *Pflüegers Arch.* 408: 565-572.

Mizumura, K., M. Minagawa, Y. Tsujii and T. Kumazawa (1990) The effects of bradykini agonists and antagonists on visceral polymodal receptor activities. *Pain.* 40: 221-227.

Mrosovsky, N., L. A. Molony, C. A. Conn and M. J. Kluger (1989) Anorexic effects of interleukin 1 in the rat. *Am. J. Physiol.* 257 (*Regulatory Integrative Com. Physiol.* 26): R1315-R1321.

Myers, A. C. and B. J. Undem (1991) Functional interactions between capsaicin-sensitive and cholinergic nerves in the guinea pig bronchus. *J. Pharmacol. Exp. Ther.* 259: 104-109.

Myers, A. C., B. J. Undem and D. Weinreich (1990) Electrophysiological properties of neurons in guinea pig bronchial parasympathetic ganglia. *Am. J. Physiol.* 259: L403-L409.

Myers, A. C., B. J. Undem and D. Weinreich (1991) Influence of antigen on membrane properties of guinea pig bronchial ganglion neurons. *J. Appl. Physiol.* 71 (3): 970-976.

Myers, A. C. and B. J. Undem (1993) Electrophysiological effects of tachykinins and capsaicin on guinea-pig bronchial parasympathetic ganglion neurones. *J. Physiol.* 470: 665-679.

Nakashima, T., T. Hori, T. Mori, K. Kuriyama and K. Mizuno (1989) Recombinant human interleukin-1 beta alters the activity of preoptic thermosensitive neurons in vitro. *Brain. Res. Bull.* 23(3): 209-213.

Nathan, P. W. and M. C. Smith (1953) Spinal pathways subserving defecation and sensation from the large bowel. *J. Neurol. Neurosurg. Psychiatry.* 16:245-256.

Nishi, S. (1986) Electrophysiological properties of sympsthetic neurons. In:*Autonomic and Enteric Ganglia.* New York: Plenum: 79-106.

Nishimura, T., J. Krier and T. Akasu (1991a) Endothelin causes prolonged inhibition of nicotinic transmission in feline colonic parasympathetic ganglia. *Am. J. Physiol.* 261 (*Gastrointes. Liver Physiol.* 24): G628-G633.

Nishimura, T., J. Krier, and T. Akasu (1991b) Endothelin modulates calcium channel current in neurones of rabbit pelvic parasympathetic ganglia. *Br. J. Pharmacol.* 103: 1242-1250.

Nishimura, T., T. Akasu and J. Krier (1992) Guanosine 3', 5'-cyclic monophosphate regulates calcium channels in neurones of rabbit vesical pelvic ganglia. *J. Physiol.* 457: 559-574.

Nishimura, T., T. Akasu and J. Krier (1993) Do depolarizing membrane oscillations regulate spontaneous discharge of action potentials in feline parasympathetic neurones. *Society for Neuroscience Abstracts.* 19: 1523.

Nishimura, T., J. Krier and T. Akasu (1993) Effect of vasoactive intestinal contractor on voltage-activeatied Ca^{2+} currents in feline parasympathetic neurones. *Am. J. Physiol.* 265 (*Gastrointest. Liver Physiol.* 28) : G1158-G1168.

Nja, A. and D. Purves (1977) Specific innervation of guinea pig superior cervical ganglion cells by preganglionic fibers arising from different levels of the spinal cord. *J. Physiol. London.* 264: 565-583.

Norberg, K. A. (1964) Adenergic innervation of the intestinal wall studied by flurescence microscopy. *Intl. J. Neuropharmacol.* 3: 379-382.

Ohlsson, K., P. Bjork, M. Bergenfeldt, R. Hageman, R. C. Thompson (1990) Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature.* 348(6301): 550-552.

Ohno, T., T. Yajima, T. Urano and K. Nakamura (1984) Interaction of prostaglandin E_2 and bradykinin in the induction of afferent spanchnic nerve discharges in cat. *Jpn. J. Pharmacol.* 34: 191-203.

Papka, R. E., J. B. Furness, N. C. Della, R. Murphy and M. Costa (1984) Time course of effect of capsaicin on ultralstructure and histochemistry of substance P-immunoreactive nerves associated with the cardiovascular system of the guinea pigs. *Neuroscience* 12: 1277-1292.

Percy, W., J. Walsh and J. Krier (1988) Morphological and electrophysiological properties of cat lumbar paravertebral neurons. *J. Autonomic Nervous system.* 24: 183-192.

Perri, V., O. Sacchi and C. Casella (1970) Electrical properties and synaptic connections of the sympathetic neurons in the rat and guinea pig superior cervical ganglion. *Pfluegers Arch.* 314: 40-54.

Petsche, U. E. Fleischer, F. Lembeck and H. O. Handwerker (1983) The effect of capsaicin application to a peripheral nerve on impulse conduction in functionally identified afferent nerve fibres. *Brain Res.* 265: 233-240.

Plata-Salamán, C. R., Y. Oomura and Y. Kai (1988) Tumor necrosis factor and interleukin-1ß: Suppression of food intake by direct action in the central nervous system. *Brain Res.* 448: 106-114.

Plata-Salamán, C. R. and J. M. H. Ffrench-Mullen (1992) Interleukin-1ß depresses calcium currents in CA1 hippocampal neurones at pathophysiological concentrations. *Brain Res. Bull.* 29: 221-223.

Plata-Salamán, C. R. and J. M. H. Ffrench-Mullen (1993) Interleukin-2 modulates calcium currents in dissociated hippocampal CA1 neurones. *Neuroreport*.. 4: 579-581.

Plata-Salamán, C. R. and J. M. H. Ffrench-Mullen (1994) Interleukin-1ß inhibits Ca^{2+} channel currents in hippocampal neurones through protein kinase C. *Eur. J. Pharmacol.* 266: 1-10.

Rada, P., G. P. Mark, M. P. Vitek, R. M. Mangano, A. J. Blume, B. Beer and B. G. Hoebel (1991) Interleukin-1 beta decreases acetylcholine measured by microdialysis in the hippocampus of freely moving rats. *Brain Res.* 550: 287-290.

Ramilo, O., M. M. Mustafa, J. Porter, X. Saez-Lloren, J. Mertsola, K. D. Olsen, J. P. Luby, B. Beutler and G. H. McCracken Jr. (1990) Detection of interleukin 1 beta but not tumor necrosis factor-alpha in cerebrospinal fluid of children with aseptic meningitis. *Am. J. Dis. Child.* 144(3): 349-352.

Regoli, D., N.-E. Rhaleb, N.-E. Dion and G. Drapeau (1990) New selective bradykinin receptor antagonists and bradykinin B_2 receptor characterization. *Trends Pharmacol. Sci.* 11: 156-161.

Rosenfeld, M. G., J-J Mermod, S. G. Amara, L. W. Swanson, P. E. Sawchenko, J. Rivier (1983) Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. *Nature* 304: 129-135.

Saha, J. K., J. N. Sengupta and R. K. Goyal (1990) Effect of bradykinin on opossum esophageal longitudinal smooth muscle: evidence for novel bradykinin receptors. *J. Pharmacol. Exp. Ther.* 252: 1012-1020.

Sawda, M., N. Hara and T. Maeno (1990) Extracellular tumor necrosis factor induces a decreased K^+ conductance in an identified neuron of *Aplysia Kurodai*. *Neurosci. Lett.* 115: 219-225.

Sawda, M., N. Hara and T. Maeno (1991) Ionic mechanism of the outward current induced by extracellular ejection of interleukin-1 onto identified neurones of *Aplysia*. *Brain Res.* 6545: 248-256.

Semba T. (1954) Intestino-intestinal inhibitory reflexes. *Jap. J. Physiol.* 4: 241-245.
Sharkey, K. A. (1992) Substance P and celcitonin gene-related peptide (CGRP) in gastrointestinal inflammation. *Annals of The New York Academy of Sciences*. 664: 425-442.

Shuttleworth, C. W., K. M. Sanders and K. D. Keef (1993) Inhibition of nitric oxide synthesis reveals non-cholinergic excitatory neurotransmission in the canine proximal colon. *Br. J. Pharmacol.* 109: 739-747.

Sims, J. E., M. A. Gayle, J. L. Slack, M. R. Alderson, T. A. Bird, J. G. Giri, F. Colotta, A. Mantovani and K. Shanebeck (1993) Interleukin 1 signaling occurs exclusively via the type I receptor. *Proc. Natl. Acad. Sci. U. S. A.* 90(13): 6155-6159.

Sims, J. E., C. J. March, D. Cosman, M. B. Widmer, H. R. MacDonald, C. J. McMahan, C. E. Grubin, J. M. Wignall, J. L. Jackson and S. M. Call (1988) cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. *Science*. 241(4865): 585-589.

Skofitsch, G., D. M. Jacobowitz (1985) Calcitonin gene-related peptide coexists with substance P in capsaicin sensitive neurons and sensory ganglia of the rat. *Peptides*. 6: 747-754.

Skok, V. I. (1973) Physiology of autonomic ganglia. First Edition by Igaku Shoin LTD. Steranka, L. R., D. C. Manning, Ch. J. de Haas, J. W. Ferkany, S. A. Borosky, J. R. Connor, J. R. Vavrek, J. M. Stewart, and S. H. Snyder (1988) Bradykinin as a pain mediator: receptors are localized to sensory neurons, and antagonists have analgesic actions. *Pro. Natl. Acad. Sci. U. S. A.* 85: 3245-3249.

Stylianou, E., L. A. O'Neil, L. Rawlinson, M. R. Edbrooke, P. Woo and J. Saklatvala (1992) Interleukin 1 induces NF-kappa B through its type I but not its type II receptor in lymphocytes. *J. Bio. Chem.* 267(22):15836-15841.

Sun, W. M., N. W. Read, A. Prior, J.-A. Daly, S.K. Cheah and D. Grundy. (1990) Sensory and motor responses to rectal distention vary according to rate and pattern of balloon inflation. *Gastroenterology* 99: 1008-1015.

Suzuki, T. (1992) Bradykinin-induced responses in hamster submandibular ganglion cells. *Bull. Tokyo Dent. Coll.* 33(1): 25-28.

Szurszewski, J. H. and B. F. King. (1989) Physiology of prevertebral ganglia in mammals with a special reference to inferior mesenteric ganglia. *Handbook of Physiology. The Gastrointestinal system I.* 519-592.

Tack, J. (1992) *Physiology of the Enteric Nervous System in the Gastric Antrum of the Guinea-Pig.* Leuven University Press, Belgium.

Undem, B., A. C. Myers, H. Barthlow and D. Weinreich. (1990) Vagal innervation of the guinea pig bronchus. *J. Appl. Physiol.* 69: 1336-1346.

Undem, B. aand D. Weinreich. (1993) Electrophysiological properties and chemosensitivity of guinea pig nodose ganglion neurons in vitro. *J. Auto. Nerv. Syst.* 44(1): 17-33.

Venkova, K. and J. Krier (1993) Stimulation of lumbar sympathetic nerves evokes contractions of cat colon circular muscle mediated by ATP and noradrenaline. *Br. J. Pharmacol.* 110:1260-1270.

Venkova, K. and J. Krier (1994) Contractions mediated by alpha 1-adrenoceptors and P2-purinoceptors in a cat colon circular muscle. *Br. J. Pharmacol.* 112:1237-1243.

Wallis, D. J. and R. A. North (1978) Synaptic input to cells of the rabbit superior cervical ganglion. *Pflügers Arch.* 374: 145-152.

Warner, S. J., K. R. Auger and P. Libby (1987) Human interleukin-1 induces interleukin-1 gene expression in human vascular smooth muscle cells. *J. Exp. Med.* 165: 1316-1311.

Watkins, L. R., E. P. Wiertelak, L. E. Goehler, K. P. Smith, D. Martin and S. F. Maier (1994) Characterization of cytokine-induced hyperalgesia. *Brain Res.* 654: 15-26.

Weems, W. A. and J. H. Szurszewski (1977) An intracellular analysis of some intrinsic factors controlling neural output from inferior mesenteric ganglion of guinea pigs. *J. Neurophysiol.* 41:305-321.

Willard, A. L. (1992) Excitatory and neurotoxic actions of platelet-activating factor on rat myenteric neurones in cell culture. *Ann. N.Y. Acad.Sci.* 664: 284-292.

Wilhelm, D. L. (1973) Chemical mediators. In *the inflammatory Process.* Edt. Zweifach, B. W., L. Grant and R. T. McCluskey: 251-301. New York: Academic Press.

Wolf, S. (1965) *The stomach.* Oxford University Press, New York.

Wood, J. D. (1981) Physiology of the enteric nervous system. In: *Physiology of the Gastrointestinal Tract* (1st ed.), edited by L. R. Johnson. New York: Raven, Vol. 1:1-38.

Wood, J. D. (1989) Electrical and synaptic behavior of enteric neurons. *Hand book of physiology. The gastrointestinal system I.* 465-517.

Wood, D. D.and S. L. Gaul (1974) Enhancement of the humoral response of T-cell-depleted murine spleens by a factor derived from human monocytes in-vitro. *J. Immunol.* 113: 925-932.

Wood, J. N., J. Winter, L. F. James, H. P. Rang, J. Yeats and S. Bevan (1988) Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. *J. Neurosci.* 8(9): 3208-3220.

Wozniak, W. and U. Skowronska (1967) Comparative anatomy of pelvic plexus in cat, dog, rabbit, macaque and man. *Anat. Anz. Bd.* 120: 457-473.

Xi, X., J. X. Thomas, Jr., W. C. Randall and R. D. Wurster (1991) Intracellular recordings from canine intracardiac ganglion cells. *J. Autonomic Nervous System.* 32: 177-182.

Yau, W. M. and M. L. Youther. (1982) Direct evidence for a release of acetylcholine from the myenteric plexus of guinea-pig small intestine by substance P. *Eur. J. Pharmacol.* 81: 665-668.

Yau, W. M., K. G. Mandel, J. A. Dorsett and M. L. Youther. (1992) Neurokinin$_3$ receptor regulation of acetylcholine release from myenteric plexus. *Am. J. Physiol.* 263 (*Gastrointest. Liver Physiol.* 26): G659-G664.

Yokota, R. and G. Burnstock. (1983) Synaptic organisation of the pelvic ganglion in the guinea-pig. *Cell Tissue Res.* 232: 379- 397.

Zeitlin, I. J., B. J. Johnson, S. Fasth, L. Hulten and C. C. Eaglesom (1978) The kinin system and non-adrenergic, non-cholinergic (NANC) nerves in the colon. *Adv. Biosci.* 22:269-270.

Zernig, G., P. Holzer and F. Lembeck (1984) A study of the mode and site of action of capsaicin in guinea pig heart and rat uterus. *Naunyn-schmiedebergs Arch. Pharmac.* 326: 58-63.