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EVALUATION OF TWO METHODS TO ELICIT SOMATIC OR VISCERAL NOXIOUS STIMULI AS PROCEDURES TO SCREEN ANALGESIC DRUGS IN RATS

presented by

Robert Alan Durham

has been accepted towards fulfillment
of the requirements for

M.S. degree in Interdepartmental
Biological Science

Donald C. Sawyer
Major professor

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EVALUATION OF TWO METHODS TO ELICIT SOMATIC OR VISCERAL NOXIOUS STIMULI AS PROCEDURES TO SCREEN ANALGESIC DRUGS IN RATS

By

Robert Alan Durham

A THESIS

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ABSTRACT

EVALUATION OF TWO METHODS TO ELICIT SOMATIC OR VISCERAL NOXIOUS STIMULI AS PROCEDURES TO SCREEN ANALGESIC DRUGS IN RATS

By

Robert Alan Durham

This study was undertaken to evaluate a modified Evans flinch-jump procedure (somatic nociception) and colorectal-balloon distension (visceral nociception) for measuring nociceptive threshold (NT) in rats. The goal was to validate these models by screening analgesic drugs such as opioids (oxymorphone and butorphanol), and non-steroidal anti-inflammatory agents (ibuprofen and Ketorolac tromethamine). Diazepam was also studied as an agent with central anxiolytic/sedative properties and no known analgesic activity to determine the selectivity for analgesia in these tests. The Flinch-jump (FJ) procedure may not represent a natural noxious stimulus only larger doses of oxymorphone (0.3 - 0.9 mg/kg) increased the NT. The colonic balloon distension (CBD) method was more sensitive and both classes of opioids increased the NT at pharmacologically relevant doses. Neither of the NSAIDs nor diazepam increased the NT in either test. The CBD was more sensitive for opioid antinociceptive effects than FJ.

This work is dedicated to my family and the close circle of friends who count me as family. Attainment of goals such as this, would not have been possible without their unending love, support, and encouragement.

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GENERAL INTRODUCTION

> "Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage...Pain is always subjective...Activity induced in the nociceptor and the nociceptive pathways by a noxious stimulus is not pain, which is always a psychological state, even though we may well appreciate that pain most often has a proximate physical cause." (Merskey, 1979).

As defined above, pain is a complex phenomenon which encompasses both physiological and psychological processes. Whereas nociception is a physiological process in which afferent neurons relay information about noxious potentially damaging stimuli from peripheral (somatic and visceral) structures to the central nervous system. The perception of these noxious stimuli as painful depends upon spinal and supraspinal integration of nociceptive transmissions leading to a unified response to threatening stimuli including: sensory/discriminatory aspects, motivational/affective and reflex autonomic adjustments. Nociceptive models have been developed with a two-fold purpose; a)to study the anatomy and physiology of nociception, and b) to evaluate analgesic

drugs. Various techniques have been used to simulate nociceptive challenges in ways that are reliable, sensitive and quantifiable. These simulations attempt to excite receptor populations involved in recognition of clinically relevant noxious events without causing tissue damage, undue pain or distress in animal subjects.

Anatomy and Physiology of Nociception

Peripheral Substrates of Nociception

Somatic nociception involves structures located in skin, subcutaneous tissue, muscle, teeth, bone, and joints which are more or less selective as transducers of noxious mechanical thermal or chemical stimuli. These transducers or nociceptors are classified according to the modality of stimulus to which they respond. High threshold mechanical (HTM) nociceptors respond maximally only to stimuli in the noxious range and as such are often referred to as nociceptive specific. C-polymodal nociceptors respond to intense mechanical, thermal and some chemical stimuli. In the rat these nociceptors constitute up to 90% of the C fiber sensory units in hairy skin (Dubner and Bennett, 1983). Thus by sheer numbers they are considered very important in the transmission of noxious stimuli. Myelinated mechanothermal nociceptors (MMTN) or A-delta heat nociceptors respond maximally to intense heat stimuli (greater than 45°C) and to intense mechanical stimuli.

Theses fibers are responsible for the first pain due to heat stimulation (Dubner and Bennett, 1983; Yaksh and Hammond, 1982). More recent studies have confirmed earlier reports (Zotterman, 1939) that both A-delta and C-fibers contribute to the dual (fast and slow) response to acute noxious stimulation of the skin (LaMotte and Campbell, 1978; Dubner et al., 1977). A-delta fibers appear to be specialized for responding quickly to mechanical and thermal insults triggering the fast, sharp, "first pain" which elicits a motor response to limit possible damage. In addition to reinforcing the responses of MMTNs in mechanical and thermal insults, C-polymodal nociceptors are sensitized by chemical mediators and are responsible for the slow burning second pain signaled by inflamed tissue. A possible benefit from this effect is the promotion of rest and protection of these tissues to allow healing (Bonica, 1990).

Visceral nociception is accomplished in a similar fashion with A-delta and C-fiber primary afferents following sympathetic and parasympathetic nerve tracts to innervate thoracic and visceral structures. However, visceral nociceptors are not necessarily excited by stimuli that would excite somatic nociceptors (Lewis, 1942). Noxious stretch or distension and some chemical stimuli evoke responses from primary afferent fibers located in viscera, whereas visceral organs are relatively insensitive to cutting or probing (Gebhart and Ness, 1991).

Smooth muscles of the hollow viscera contain mechanoreceptors connected to both A-delta and C fibers which join the vagal parasympathetic supply. These non-specific mechanoreceptors or tension receptors are active during normal non-painful functions. Nerve activity dramatically increases in response to isometric contractions which are usually associated with pain (Leek, 1972). These findings led Iggo (1974) to conclude that these non-specific tension receptors might have a dual function. At low levels of activity, these receptor-afferent units regulate visceral and vascular reflexes, while at higher levels of activity, they function as nociceptor-afferent units. However, conclusive evidence has been shown that noxious stimuli (pain) from the alimentary tract travel via splanchnic and other sympathetic nerves (Cerevero and Morrison, 1986). Splanchnic afferent mechanosensitive receptive fields are distributed along the superior mesenteric artery, vessels in the mesentery, and walls of the viscera (Morrison, 1977). These receptors are slowly adapting A-delta and C fibers which respond to light mechanical stimuli, e.g. tension applied to mesentery and visceral peritoneum, smooth muscle contraction, and visceral distension. These fibers are thought to be directly involved in pain because they respond to stimuli known to evoke painful sensations and follow sympathetic pathways with cell bodies in the dorsal root ganglia (Morrison, 1977). It has been suggested that, since

these receptors lack specificity, they might encode information from noxious stimuli based on intensity (Cervero, 1983; Cervero and Morrison, 1986). Thus the non-specific tension receptors which join the vagus are not likely to be directly involved in nociception and increased activation may only be a coincidence of increased tension or stretch.

In contrast to somatic sensations visceral sensations are poorly localized. It has been estimated that visceral afferent fibers constitute less than 10 % of the afferent fibers in dorsal roots. "Thus the visceral sensory field of each dorsal root is formed by extensive branching of relatively few parent afferent fibers so there is up to 100% overlap between the receptive fields of adjacent roots" (Bonica, 1990). There is a much higher proportion of small fibers in visceral primary afferents compared to cutaneous primary afferents. Among group A fibers, A-deltas predominate and the ratio between A and C-fibers is 1:8 or 1:10 compared to the ratio at the dorsal root which is 1:2 (Jänig and Morrison, 1986). The primary afferents from the descending colon and rectum pass through the pelvic nerves and enter the spinal cord via ventral and some dorsal roots of S2, S3, and S4 (Cervero and Morrison, 1986).

Dorsal Horn

Two types of nerve cell bodies can be morphologically

differentiated in the dorsal root ganglion (Lawson, 1979). Although there are cells which do not rigidly fit into either category type A are large lightly staining cells and type B are small darkly staining cells. Initially larger A fibers were thought to be associated with type A cell bodies and Small diameter A-delta and C fibers are often associated with type B cell bodies (Lawson et al., 1984). There is more recent conflicting evidence whereby fiber type is (Harper and Lawson, 1985) and is not (Hoheisel and Mense, 1986) correlated with cell body type. Correlations of this type would allow one to readily distinguish between fiber types based on cell body type.

The central projections of primary nociceptive afferents synapse in the dorsal horn. The spinal cord grey matter is conventionally divided into laminae based on an ultrastructural organization of neurons (Rexed, 1954). Although laminae do not represent strict borders, lamina I is called the marginal zone, lamina II the substantia gelatinosa, laminae III - V the nucleus proprius, and lamina VI the base of the dorsal horn (Bonica, 1990). Laminae I and V are the most prominent sites where primary nociceptive afferents synapse in the dorsal horn. Many cells in lamina I respond only to noxious stimuli and thus are known as nociceptive-specific (NS) (Cervero et al., 1976). A larger portion of cells in this lamina are projection cells. They make up both long ascending pathways and shorter

intersegmental connections involved in the organization of sensory transmission (Basbaum, 1984; Yaksh and Hammond, 1982). Most lamina V cells are multireceptive or wide dynamic range (WDR) neurons that respond to a variety of inputs; low to high threshold mechanical, thermal, and chemical stimuli from A-delta and C-fiber primary afferents (Wall, 1989). Some lamina V cells respond to input from both visceral and cutaneous nociceptors which may serve as the anatomical substrate for referred pain (Pomeranz et al., 1968). Axons of Cells in this lamina also make significant contributions to the ascending nociceptive pathways (Cervero and Morrison, 1986).

The substantia gelatinosa (lamina II) is dominated by small neurons with dendrites branching in the rostral caudal plane. In this lamina, stalked cells and islet cells are two prominent cell types considered important as dorsal horn nociceptive local-circuit neurons (Gobel, 1978). Stalked cells are thought to function as excitatory local circuit neurons (Gobel, 1978), dendrites of these cells receive synapses from primary afferents and possibly descending serotonergic axons (Dubner and Bennett, 1983). Islet cells are thought to function as local inhibitory interneurons (Gobel, 1978). Some islet cells are GABAergic while others are enkephalinergic and like stalked cells, islet cells also receive input from descending serotonergic axons (Dubner and Bennett, 1983; Gobel, 1978). The substantia gelatinosa is

not a closed system and some interneurons project into surrounding laminae and also include projections in the long ascending tracts (Willis, 1985).

Neurochemical transmission and modulation of nociceptive input in the dorsal horn is a complex and intricate process that is not fully understood. Neurochemicals such as 5-Hydroxytryptamine (5-Ht), gamma-aminobutryic acid (GABA) and the peptides (substance P, vasoactive intestinal peptide (VIP), calcitonin gene related peptide (CGRP), cholecystokinin (CCK), somatostatin, neurotensin, and endorphins) found in the dorsal horn have been ascribed various excitatory and inhibitory roles in nociception. Direct inhibition of spinothalamic tract cells in lamina I has been shown with iontophoresis of 5-HT (Willcockson et al., 1984). Electrical stimulation of the nucleus raphe magnus (NRM) inhibits nociceptor-specific (NS) and multi-receptive (WDR) lamina I neurons (Miletic et al., 1984, Ruda et al., 1982). NRM stimulation also inhibits stalked cells of lamina II. However, deeper islet cells have few 5-Ht contacts and are not inhibited by NRM stimulation. Both $GABA_a$ and $GABA_b$ receptors have been seen on presynaptic aspects of A-delta and C-fiber primary afferents (Barber et al., 1978; Patrick et al., 1983). $GABA_a$ receptors are associated with bicuculline sensitive Cl^- channels, while $GABA_b$ receptors mediate a decrease in Ca^{2+} conductance (Desarmenien et al., 1980). Increased Cl^- conductance

and/or decreased Ca^{2+} conductances have been implicated as the ionic basis for inhibitory control in synaptic transmission. Substance P, CCK, VIP, and somatostatin have all been ascribed excitatory roles in the transmission of nociceptive information (Otsuka et al., 1982; Leah et al., 1985). Neurotensin produces a dose dependent excitation of spinal neurons (Stanzione and Zieglgansberger, 1983) and is also present in islet cells (Seybold and Elde, 1982) which makes this a possible candidate for nociceptive transmission at the level of substantia gelatinosa interneuronal circuits.

Considerable evidence supports a modulatory role in nociception for endorphins. Opiate binding sites are associated with primary afferent fibers (Fields et al., 1980). The prototype mu peptide agonist enkephalin hyperpolarizes dorsal horn neurones through an increase in K^{+} conductance (Werz and MacDonald, 1982a, b; Yoshimura and North, 1983). Additionally, enkephalins have been shown to block Ca^{2+} current which produces the broad action potentials of C-type DRG neurons in culture (Mudge et al., 1979). Similar results have been shown with kappa ligands, such as dynorphin (Chavkin et al., 1982), which inhibit populations of DRG neurons (MacDonald and Werz, 1986). Opiate agonists which interact with the kappa receptor may decrease the inward Ca^{2+} current necessary for synaptic transmission via a direct mechanism (Werz and MacDonald,

1983). Opiates block substance P release following high-intensity stimulation of peripheral nerves (Yaksh et al., 1980). Taken together, the function of the spinal cord dorsal horn is more than just a simple relay station but performs a complex integration of neuronal signals. The output is then passed on to spinal and supraspinal regions to result in complex somatic and autonomic responses to noxious stimuli (Taylor and Pierau, 1991).

Ascending Nociceptive Pathways

The major ascending tracts involved in nociception follow the anterolateral white matter of the spinal cord (often referred to as ventrolateral in animals). Three distinct tracts; the spinothalamic (STT), spinoreticular (SRT), and the spinomesencephalic (SMT) make up the anterolateral fasciculus (ALF) and represent the primary paths for nociceptive information from the spinal cord to supraspinal regions. The spinothalamic tract arises from cells in dorsal horn laminae (Giesler et al., 1981a). These axons decussate within the spinal cord and travel within the anterolateral white matter to the brain (Willis et al., 1979; Jones et al., 1985). Evidence for involvement of the STT in nociception comes from observations following experimental or clinical lesions in the anterolateral (ventrolateral) region of the spinal cord. Experimental lesions of the STT diminished responses to noxious stimuli

in rats (Peschanski et al., 1986). In lesions of the anterolateral tracts, humans report that pain sensation is absent below the segmental level of the lesion on the contralateral side of the body (White and Sweet, 1969). Further compelling evidence was reported in an individual case history in which pain sensations remained when all but the anterolateral quadrant of the spinal cord had been transected (Noordenbos and Wall, 1976).

Spinothalamic axons terminate mainly in the ventrobasal complex (Peschanske et al., 1983). A subgroup of cells from laminae I and V which project directly to the ventroposteriolateral nucleus and medial part of the posterior thalamus are referred to as the neospinothalamic tract (Kerr and Lippman, 1974; Brown, 1981; Yaksh and Hammond, 1982). These fibers then synapse and project to somatosensory cortex. The remainder of the STT branches to form the paleospinothalamic tract (paramedial ascending system), which also includes SRT and SMT axons that project to nuclei of the medullary reticular formation, to synapse and project onto periaquaductal grey (PAG), hypothalamus, medial intralaminar thalamic nuclei, and limbic structures.

Three functional types of neurons have been represented by axons in the spinothalamic tract. Nociceptive specific (NS) neurons located primarily in lamina I give a slowly adapting response to high threshold noxious mechanical and thermal stimuli. Some reports indicate that nearly two-

thirds of STT neurons that project exclusively to medial thalamus are NS neurons (Giesler et al., 1981b). WDR neurons respond in a frequency-dependent fashion to stimuli of increasing intensity. These neurons, with cell bodies located primarily in lamina V and a few in lamina I (Dubner, 1985), exhibit a sustained discharge to pressure and a rapid adaptation to noxious thermal and chemical stimuli. WDR neurons also receive converging input from cutaneous, visceral, and muscle primary afferents which provides a mechanism for referral of visceral pain to somatic receptive fields (Willis, 1985). Cell bodies of Narrow dynamic range neurons located in laminae IV and V also contribute to the STT. These neurons respond only to innocuous tactile and thermal stimuli.

Spinal projection neurons to the pontomedullary reticular formation are known as the spinoreticular tract (SRT), (Mehler, 1969). Many of these neurons identified by antidromic stimulation are classified as nociceptive and have characteristics similar to either WDR or NS neurons described above (Willis, 1985). Double labeling experiments show that some SRT neurons project to both the thalamus and reticular formation (Giesler et al., 1981b; Keveter and Willis, 1982). This evidence seems to be in agreement with the earlier assertion that SRT neurons are likely to trigger arousal and to contribute to motivational-affective aspects of nociception as well as autonomic, motor, and somatic

reflexes (Bowsher, 1976).

In rats, the spinomesencephalic projection from lamina I is primarily to PAG and cuneiform nucleus (Swett et al., 1985). These cells located in Lamina I respond exclusively to noxious stimuli (Hylden et al., 1986a). Electrical stimulation near the PAG in humans causes the sensation of diffuse pain referred to the central part of the body or a feeling of fear (Nashold et al., 1969). Stimulation of PAG has also resulted in analgesia as well as aversive reactions (Willis, 1982). Thus investigators have speculated that midbrain projections are involved in affective aspects of pain, autonomic responses, and pain modulation (Hylden et al., 1985, 1986a,b).

Spinocervical and postsynaptic dorsal column tracts have also been implicated in nociception. Neurons in the upper cervical cord of the rat represent a true spinocervical tract and many of these cells respond to noxious stimuli even in the presence of anesthesia (Giesler et al., 1979b). In humans, a role for these pathways in nociception has been determined from evidence that chronic pain often returns in the years or months following an initially successful cordotomy of the anterolateral quadrant of the spinal cord (White and Sweet, 1969). The remaining spinocervical and postsynaptic dorsal column tracts are believed to conduct nociceptive information in the absence of a functional anterolateral quadrant. It is not clear

whether the return of sensation is adaptive, i.e. these tracts assume a nociceptive function in response to cordotomy, or if these tracts represent additional nociceptive pathways in normal individuals.

Supraspinal Structures and Nociception

The reticular network of neurons is organized to distribute information quickly to multiple supraspinal sites from the spinal cord, to thalamus, limbic structures, and cortex (Bowsher, 1976; Casey, 1971; Casey, 1980a). Physiologic studies show that the reticular formation neurons can mediate motor, autonomic and sensory function (Casey, 1980b). Selective lesions of the reticular formation attenuate aversive quality of noxious stimuli without affecting discriminatory aspects of nociception (Casey, 1980b). Similarly, the effect of narcotic analgesics on reticular formation also reduces suffering in response to clinical pain without affecting the ability to recognize noxious stimuli (Casey, 1980b).

Several regions in the thalamus receive input from ascending nociceptive tracts. The ventrobasal complex consisting of ventral posterior thalamic regions divided into medial and lateral nuclei, is thought to be involved in sensory discriminatory functions. The STT from laminae I and V end in the ventroposterolateral nuclei (VPL). These nuclei respond primarily to mechanical stimulation (see

Willis 1985 for references) and are somatotopically organized with receptive fields on the contralateral side of the body (Willis, 1985; Willis and Coggeshall, 1978; Netter, 1983). The VPL then sends projections to and receives projections from somatosensory cortical regions, SI and SII (Willis, 1985).

The ventroposteromedial nucleus (VPM) receives input from trigeminal subnucleus caudalis, and sn. interpolaris. Cells in the VPM exhibit a high degree of somatotopic organization and have small receptive fields on the contralateral side of the face. Like the VPL the major portion of cells respond to low threshold mechanical stimuli; however, some neurons respond as NS or WDR as well (Willis, 1985). VPM fibers project to and from the facial region in the somatosensory cortex, i.e. the lateral part of the cerebral convexity in the postcentral gyrus (Jones and Friedman, 1982).

The posterior group of thalamic nuclei also receive spinal somatic sensory input from the STT. However, this region of the thalamus lacks the somatotopic organization found in the ventrobasal complex (Willis, 1985). The receptive fields are bilateral and large with many cells responding only to noxious stimuli (Poggio and Mountcastle, 1960). Lesions in the posterior group region have been reported to provide pain relief if the lesions also include medial thalamic structures (Willis, 1985).

The role ascribed to medial and intralaminar thalamic nuclei is related to motor and emotional reactions and aversive drive. Cells in these nuclei respond most vigorously to noxious stimuli (Casey, 1966). These cells are not somatotopically organized and the receptive fields are large and bilateral (Dong and Wagman, 1976). Lesions in this region relieve the affective dimensions of pain while preserving the somatosensory discriminatory aspects. Although there are apparently no direct connections with cortical regions, electrical stimulation of this region results in widespread cortical effects and thus these nuclei may be functioning as part of the diffuse reticular activating system (Bonica, 1990).

Studies of the hypothalamus and limbic structures have demonstrated a neural basis for aversive drive and affect and descending modulatory influences which comprise the motivational dimensions of nociception. Electrical stimulation in the lateral hypothalamus elicits a reward response but in posterior medial hypothalamus elicits escape behavior. Similarly, electrical stimulation of hippocampus, fornix and amygdala evoke escape (Olds and Olds, 1963). In the lateral central grey region there is a correlation between current threshold of brain stimulation to block pain, and self stimulation threshold current perceived as reward (Dennis et al., 1980).

Experimental Models of Nociception

Many investigators have defined several criteria by which we can critically evaluate the various experimental methods to study nociception and antinociceptive agents (Beecher, 1957; Lineberry, 1981; Wood, 1984; Raffe, 1992).

1. Test Quantitation. Parameters must be quantifiable and controlled with precision to minimize experimental variability.

2. Test validity. Stimulus should be relevant to the question being asked. Since different classes of analgesic drugs have very different mechanisms of action it seems reasonable that the efficacy of these analgesics may vary depending on the noxious stimulus. In terms of clinical usefulness one cannot rely on a single nociceptive test to determine the efficacy of a drug.

3. Test reliability/repeatability. Is the test response reproducible if measured frequently in a given day or from day to day? Since noxious stimuli are often capable of tissue damage, frequent stimulation is problematic in that tissue damage incurred may alter the response characteristics of the tissue over time (increase sensitivity or desensitize).

4. Test simplicity. Does the procedure use a standard defined noxious stimulus and measure a well defined animal response? Stimuli should as nearly as possible simulate natural stimuli and involve only those peripheral systems normally involved in nociception.

5. Test sensitivity. Can different analgesic doses of a drug be discriminated and can the procedure detect a partial agonist or weak analgesic response?

Although no single nociceptive model rigorously meets all of these criteria, a study of the "ideal method" is helpful to understand the strengths and weaknesses of the individual models under consideration.

The various models of animal nociception are usually categorized based on the noxious stimulus used. In terms of assessing the effects of antinociceptive agents and other experimental manipulations, most tests regardless of noxious stimulus measure either nociceptive threshold or nociceptive tolerance. Nociceptive threshold (NT) determination is the most commonly used method for studying the efficacy of antinociceptive drugs (Lineberry, 1981). In animals NT is usually defined as the minimum stimulus necessary to elicit a predictable end-point response. Within the context of the above criteria this method has several advantages and some disadvantages. NT is easily defined and provides a convenient measure by which analgesic drugs or other

manipulations can be compared. Additionally, by using the minimum stimulus necessary the possibility of inflicting suffering or causing tissue damage is reduced.

One of the major concerns raised with NT determinations is that of validity. Depending on the noxious stimulus used and the end-point observed the NT may reflect only segmental reflex activity (Raffe, 1992). In humans NT is equated with "first pain" and associated with sharp, short latency sensations, while more intense stimuli represent the long latency "second pain" thought to be more relevant to clinically observed pain and associated with intense emotional responses (White and Sweet, 1969). A second inherent problem of NT measurement is found in stimulus presentation. If threshold is determined by limits, i.e. gradually increasing the stimulus intensity from zero to the defined end-point in order to prevent bias, one should also present the stimuli in descending order and determine the point where the stimulus is no longer perceived. In nociceptive models this is usually not practical due to the risk of tissue damage and associated tissue changes leading to sensitization or desensitization. Thus a constant error may introduced by only using an ascending series of stimuli to determine NT (Lineberry, 1981).

Somatic cutaneous stimuli

Cutaneous nociception has been studied using mechanical, thermal, and electrical stimuli. The

appropriate choice of stimulus may depend upon the exact nature of the experimental situation and the responses to be measured. Mechanical or electrical stimuli are the easiest to control for rapid onset whereas with chemical or thermal stimuli a rapid onset is difficult to achieve. Similarly thermal or mechanical noxious stimulation is difficult to control in a behaving subject and consideration must be given to the type of restraint necessary.

A technique using electrical stimulation has been developed in rodents and is known as the flinch-jump procedure (Evans, 1961, 1962). In this model, a 1 sec. constant current shock is applied to the grid floor of the animal cage while the subject's behavior is noted. The current level is changed (increased or decreased) in 0.1 mA increments after each stimulus presentation following an ascending or descending order of stimulus intensity according to the method of limits for threshold determination. Two distinct thresholds can be determined using this method. The first response is a flinch or crouching behavior. At higher levels of stimulation the subject jumps to avoid the shock. The assumption is that the flinch threshold may reflect the first perception of pain in humans, whereas the jump threshold may be analogous to second pain. A variety of analgesic and non-analgesic drugs have been tested using this model and only analgesic drugs increase the jump threshold. None of the drugs tested

altered the flinch response (Evans, 1964).

Subsequently a modification of the Evans flinch-jump method has been used to measure hyperalgesia in morphine dependent rats (Tilson et al., 1973). The modifications to the original Evans flinch-jump method included a dramatic reduction in the number of shocks delivered to the subject. Thresholds were determined by gradually increasing the shock current from (0 - 0.3 mA) in 0.05 mA increments; above 0.3 mA current was increased in 0.02 increments until a positive response was elicited. Positive response was defined as a rapid withdrawal of both front paws from the grid associated with a vocalization. Each animal served as its own control and the threshold represented the mean of three pre-drug determinations. Apparently, humane concerns dictated that stress induced in the animals should be reduced (fewer shocks to determine threshold) and this outweighed the possibility of inducing bias by not following the method of limits used by Evans to determine flinch-jump thresholds. Tilson et al. (1973) were able to demonstrate morphine analgesia, tolerance during chronic morphine, and a hyperalgesia upon withdrawal of morphine using the modified Evans flinch-jump protocol.

Visceral stimuli

Visceral nociception is clinically important yet poorly understood compared to cutaneous nociception. This lack of

understanding is probably due to the fact that there are relatively fewer reliable animal models of visceral nociception. Viscera often appear insensitive to stimuli which are noxious in somatic tissues, and nociceptive responses in viscera require selection of the appropriate stimuli (Lewis, 1942). Painful visceral sensations in man are more closely related to pressures rather than volumes of gut distension (Goligher and Hughes, 1951). Mechanical distension of hollow organs has been shown to be a reliable and effective noxious visceral stimulus in several species: Horses(Lowe, 1969, 1970; Pippi and Lumb, 1979) dogs (Houghton et al., 1991; Sawyer et al., 1991; Sawyer et al., away) cats (Sawyer and Rech, 1987; Sawyer et al., 1990; Durham et al., 1992; Sawyer et al., 1992b) and rats (Rech et al., 1987; Ness and Gebhart, 1988; Colburn et al., 1989).

STATEMENT OF PURPOSE

This study was undertaken to evaluate two methods; a modified Evans flinch-jump (somatic nociception) and colorectal-balloon distension (visceral nociception) for measuring nociceptive threshold (NT) in rats. These were used to characterize the selective analgesic properties (visceral or somatic) of two opioid drugs, butorphanol and oxymorphone. Additionally to validate these models a variety of drugs with prominent non-steroidal anti-inflammatory, or other central nervous system activity were tested.

MATERIALS AND METHODS

Subjects

Approval for use of vertebrate species was granted by the All University Committee for Animal Use and Care. All subjects in these experiments were Sprague Dawley male rats weighing between 300-600 grams. They were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Rats were housed in plexiglass cages with wire tops in a room with a 12 hour light/dark cycle and controlled temperature (72 ± 2 °F)and humidity (40-70%).

Flinch-jump

Equipment

The equipment consisted of a plexiglass box (6.75" x 8.25" x 14") with a grid floor. Electric current was delivered to the grid by means of a constant-current shocker-scrambler (Lafayette Instruments Co. model A-615C). Stimulus intensity was regulated with a variable resistor.

Training

This procedure required very little training, however rats were trained two or three times a week on days not involved in actual experiments. Training consisted of placing subjects in the grid-floor cage and running a 20-30 minute no-shock dummy session to extinguish tendencies to

develop conditioned responses to auditory cues and the surroundings of the experimental paradigm.

Protocol

Rats were placed in a grid-floor cage and somatic NT was determined by applying brief 0.5 sec. pulses of constant current electric shock to the grid. The stimuli were presented in ascending order with intensity increasing in 0.05 mA increments up to the level of first predictable response; about 0.35 mA in Sprague-Dawley males which was the minimum threshold detectable by the observer. At this point, the observer noted a rapid withdrawal of both forepaws (by the rat) from the grids or the "flinch" response. Many subthreshold stimuli were given to avoid stress and extinguish tendencies of rats to develop a conditioned response to non-painful parameters of the procedure (auditory cues).

Data

Data presented from these experiments represented the somatic flinch-Jump NTs in mA expressed as a percentage of the maximum possible effect (MPE). The formula for arriving at the MPE is described in the statistics section (page 37).

Colorectal-balloon distension

Equipment

Equipment used for these studies included the following: (1) cloth hand towels (30 x 50 cm), and metal spring pinch clips (ACCO Inc.) for restraint; (2) colonic balloon catheter; (3) inflation/deflation system (Figure 1); (4) custom made strain gauge clip to measure pseudoaffective behavioral responses; and (5) Grass Model 7D ink writing oscillograph.

The colonic balloon catheter was specifically developed for use in colorectal distension studies and manufactured by Bivona Inc. (Gary, IN). The device is a 20 cm long 6 fr. catheter with a luer loc® connector and a 25 X 8 mm balloon incorporated into the tip (Figure 2).

The strain gauge clip (Figure 3) was designed and built by Thomas Adams, Ph.D. (Michigan State University Department of Physiology). Two microfoil strain gauges (Omega Engineering, Inc.) were bonded to spring steel (0.05mm thick). When the clip arms were moved the spring steel flexed and induced a stretch or strain in the gauges. The gauges were connected to the oscillograph such that they formed a variable resistance arm in the bridge of the preamplifier. Thus, strain in the clip was transduced as alterations in resistance. The strain gauge clip was calibrated by using a vernier caliper to displace the arms of the clip known distances. These resistance changes were

then recorded as voltage calibrated pen deflections on the oscillograph (Figure 4).

Training

Four to six weeks prior to beginning of studies rats were trained and handled on a five day per week schedule. Gradually they were restrained in the towels for longer and longer periods until they became accustomed to the 45 to 60 minute sessions of restraint. Honey Nut Cheerios (General Mills Inc.) were used as treats for positive reinforcement of this behavior. After animals were fully trained, colonic balloons were inserted but not inflated during at least one of the five weekly training sessions to assure that rats would become accustomed to the presence of the balloon.

Protocol

After rats were fasted for 24 hours they were brought to the laboratory, wrapped in a towel and three spring clips were used to hold the towel in place. The microfoil strain gauge clip (Figure 3) was fitted around the rat's abdomen to measure pseudoaffective behavioral responses to colorectal distension. A balloon catheter (Figure 2) was then inserted into the colon by passing it thorough the anal opening. The inflation system (Figure 1) was used to inflate the balloon to a pre-determined pressure. Intensity of the distension stimulus was determined by pumping room air into the reservoir. System pressure was read from the calibrated manometer. The balloon was rapidly inflated in less than

0.5 seconds by opening the stop-cock to the reservoir. There was no observable pressure drop since the volume of the 1 liter reservoir is much larger than the 2 to 10 ml balloon volume. The stimulus was terminated by opening the stop-cock to the inverted graduated cylinder. Water in this cylinder was displaced by incoming air allowing the balloon volume to be measured. When a pressure pulse distended the colon sufficiently to reach the minimum nociceptive threshold, the rat responded by increasing tone of abdominal muscles referred to as a "guarding" response. These purposeful movements were recorded on the oscillograph.

Data

Data from these experiments represented the visceral NT balloon pressure in mmHg and were expressed as a percentage of the maximum possible effect (MPE). The formula for arriving at the MPE is described in the statistics section (page 37).

The objective oscillograph recordings of responses to CBD were converted to digital records using a digitizer tablet (Neumonics 2200), and SigmaScan™ software (Jandel Scientific Inc.). A 10 sec. interval of data (5 sec. before to 5 sec. after the 1 sec. CBD stimulus) were analyzed for maximum height from baseline and total area under the curve (Figure 5 and Table 1). Both of these measurements were made to arrive at the most reliable method for differentiating between a positive nociceptive response and

occasional negative non-response gross movements.

Table 1. Comparisons of area under the curve and maximum change in height; positive responses at nociceptive threshold and negative non-response gross movements.

POSITIVE		NEGATIVE	
Area mm^2	Height mm	Area mm^2	Height mm
0.86 ± 0.42	5.4 ± 0.7	0.17 ± 0.10	0.82 ± 0.36

Volume data were obtained by collecting air released from the balloon after deflation in an inverted graduated cylinder (Figure 1). This served as a means of checking to insure that the pressure induced by CBD was not distending the colon beyond a 10 ml volume which was defined as the cut-off maximum volume to prevent tissue damage.

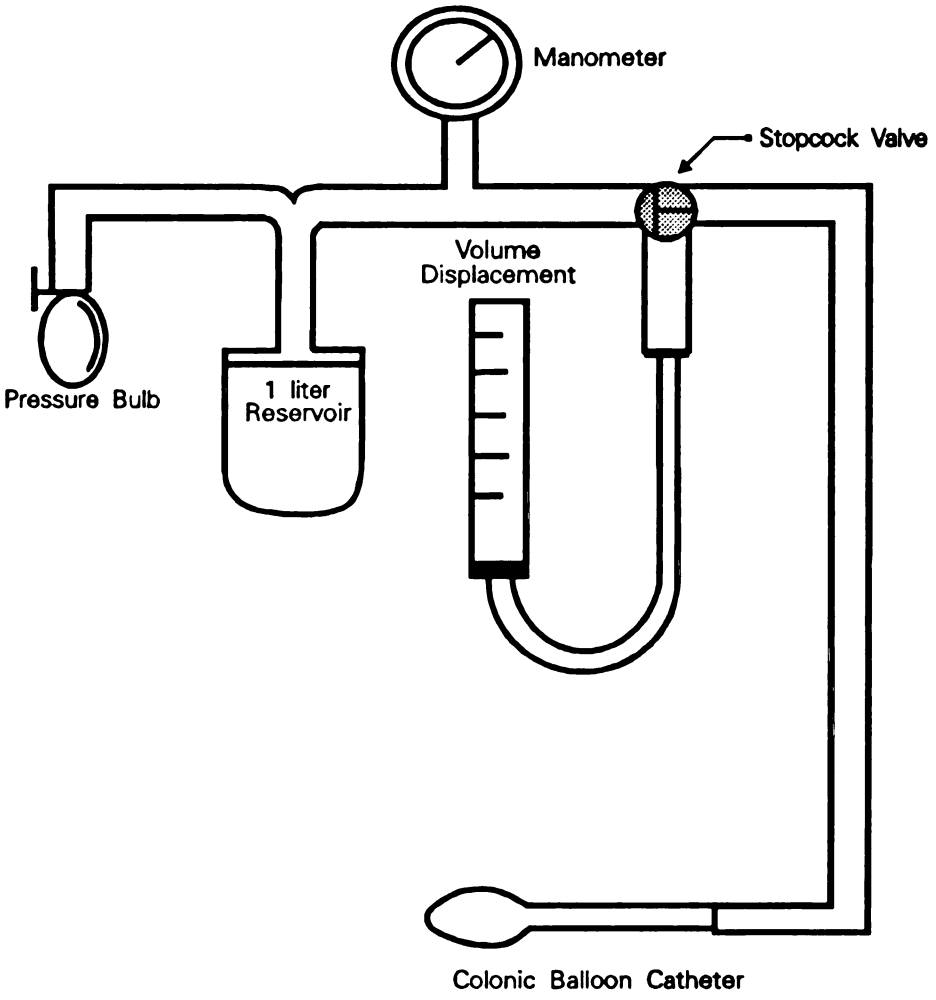

Figure 1. Inflation/deflation system used to rapidly inflate the colorectal balloon and measure the volume of air upon deflation.

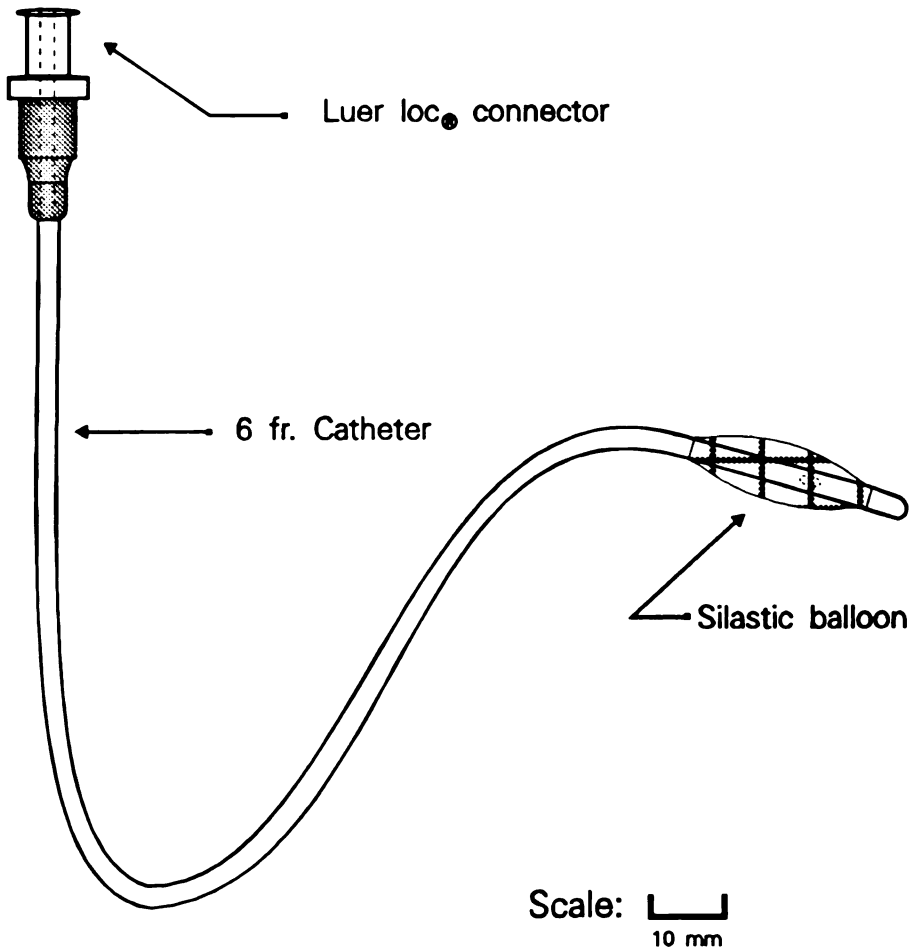

Figure 2. The colorectal balloon catheter used to distend the colon in the colorectal balloon distension procedure. Overall length is 20 cm and the balloon dimensions are 25 X 8 mm.

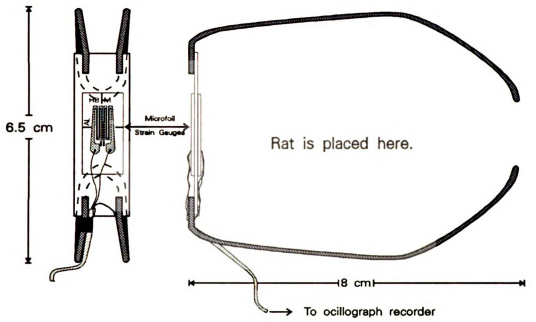

Figure 3. Strain gauge clip used to measure behavioral responses to colorectal distension.

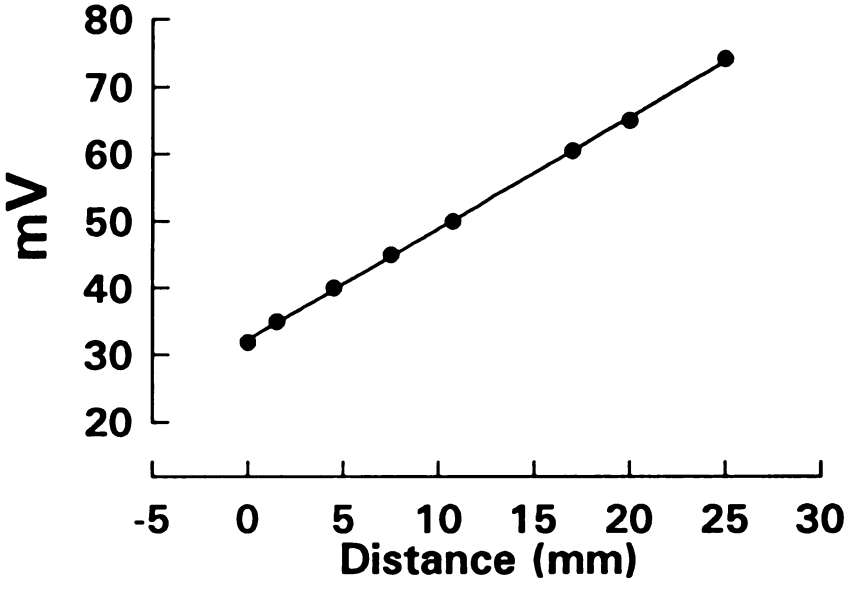

Figure 4. Strain gauge calibration curve.

mV = millivolts

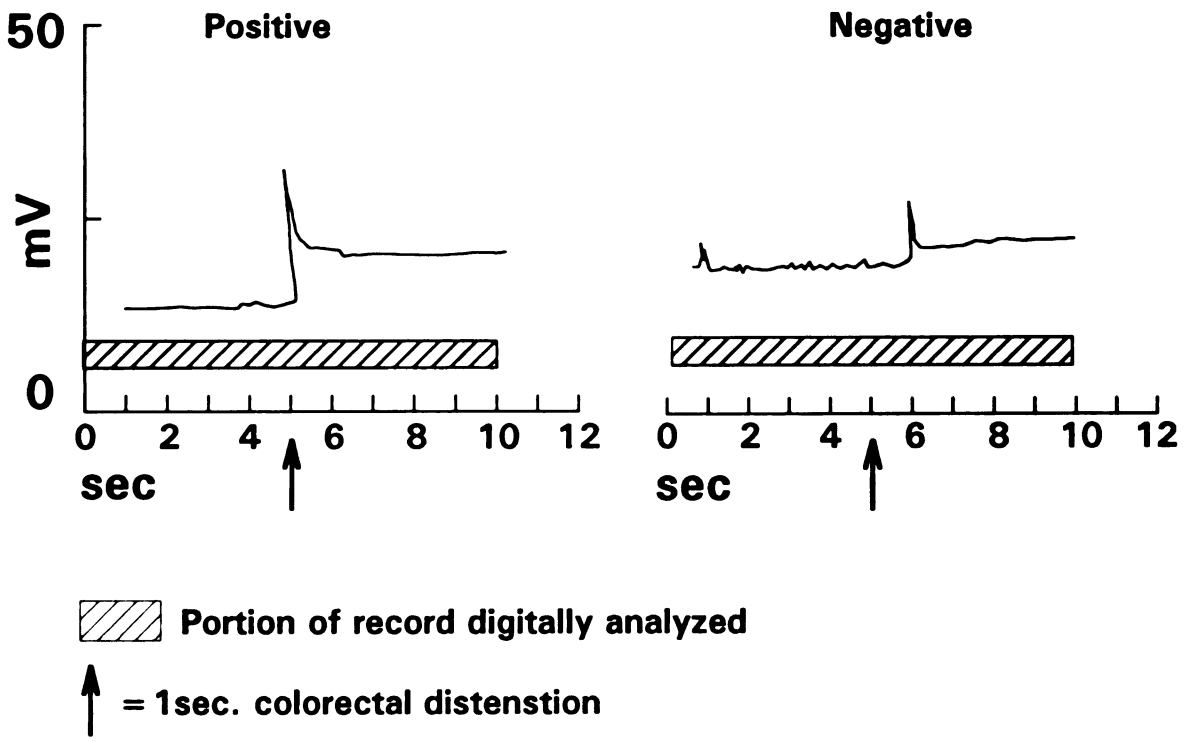

Figure 5. Two digitized oscillograph records; a positive response at nociceptive threshold and a negative non-response gross movement.

mV = millivolts

Drugs

The drugs selected for these studies were chosen for either known analgesic efficacy or other significant central effects to test the selectivity of the two nociceptive tests for preferentially selecting for antinociceptive activity. After FJ or CBD thresholds were determined rats were given subcutaneous (SQ) injections of a test drug or equal volumes of saline or vehicle as a controls. At 15, 30, 60, 90 and 120 minute intervals after drug administration, the NTs were redetermined. These studies were performed in a randomized and blinded format.

1. Oxymorphone (mu agonist) with approximately 170 times the potency of morphine given SQ in rats (see Kissel et al., 1961; Steinfels and Cook, 1986). Dosages of 0.1, 0.3, 0.6, and 0.9 mg/kg were tested in the FJ procedure. These dosages and additional dose of 0.03 mg/kg were also evaluated in CBD.

2. Butorphanol a mixed kappa/mu agonist-antagonist was tested at 0.025, 0.05, 0.1, 0.25, 0.5 and 1.0 mg/kg dosages in both FJ and CBD procedures.

3. Ibuprofen a non-steroidal antiinflammatory analgesic (NSAID), was suspended at concentrations of 10, 20 and 40 mg/ml in a 2% methylcellulose and

Tweens solution. Rats were given 1 ml SQ injections of the suspension or vehicle (2 % methylcellulose and Tweens).

4. Ketorolac tromethamine an injectable NSAID clinical use has shown efficacy near that obtained with morphine. A 30 mg/kg dosage was tested in both FJ and CBD models.

5. Diazepam, an anxiolytic/muscle relaxant with no known analgesic activity was chosen for a prominent central sedative effects to test the selectivity of these models. A 1.0 mg/kg dosage was evaluated in both FJ and CBD procedures.

6. Naloxone, a mu selective pure opioid antagonist, was given after pre-treatment with either 0.05 mg oxymorphone/kg or two doses of 0.1 mg butorphanol/kg in the CBD test. Alterations in antinociceptive effects were evaluated after 0.01, 0.05, 0.1 and 0.4 mg naloxone/kg or an equal volume of saline.

Statistics

A randomized block analysis of variance (ANOVA) and Tukey's test were used to determine significant differences

($p < 0.05$) in nociceptive threshold between drug doses at each time point. Probit analysis was employed to determine the ED_{50} and 95 percent confidence intervals for oxymorphone in the FJ procedure and CBD.

The nociceptive threshold data for either the FJ or CBD are represented graphically as percent maximum possible effect (MPE); (Harris and Pierson, 1964).

$$MPE = \frac{PDn - PDC}{PDn - MAX} x100$$

Where the NT at post drug time = n minutes (PDn) is converted to a percentage of the maximum possible range from the baseline pre-drug control nociceptive threshold (PDC) to the cutoff maximum stimulus limit (MAX).

RESULTS

The time course for changes in FJ threshold after oxymorphone is shown in Figure 6. At the 30 minute period only 0.9 mg oxymorphone/kg was significantly different from saline control. This dosage was also different from 0.1 and 0.3 mg/kg dosages. At 60 minutes, 0.9 and 0.6 mg oxymorphone/kg were significantly different from all other dosages and saline. At later time points, 90 and 120 minutes, the flinch-jump NT for 0.9 mg/kg dosage remained significantly greater than the NT for 0.3 mg/kg. These dosages were evaluated in the CBD model and the time courses for elevations of the NT are shown in Figure 7. An additional dose of 0.03 mg oxymorphone/kg was included because dosages at 0.3 and above were maximally efficacious and this additional dose was necessary to complete the dose-response curve. At the 60 minute time point, the NT for 0.1 mg oxymorphone/kg was also significantly different than 0.03 mg/kg and saline. A comparison of oxymorphone dose-response curves was made for both FJ and CBD at 60 minutes (Figure 8). The calculated ED_{50} (95% confidence limits) for oxymorphone in the FJ procedure was 0.55 (0.23 - 1.34) mg/kg while the ED_{50} in the CBD was 0.07 (0.03 - 0.16) mg/kg. The increase in NT following 0.05 mg oxymorphone/kg was reversed when rats were treated with 0.1 mg naloxone/kg at 45 minutes

(Figure 9). However, 0.01 mg naloxone/kg failed to reverse the effects of oxymorphone.

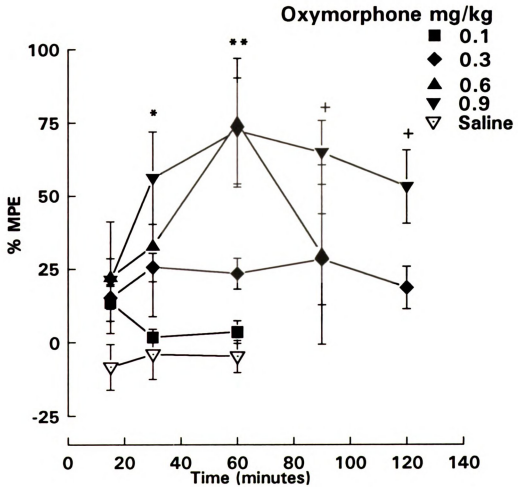

Figure 6. Flinch-jump threshold after oxymorphone administration.

*0.9 mg/kg significantly different ($p < 0.05$) from 0.3, 0.1 mg/kg or saline control. **0.9 or 0.6 mg/kg significantly different from all other doses. + 0.9 mg/kg significantly different from 0.3 mg/kg. MPE = maximum possible effect.

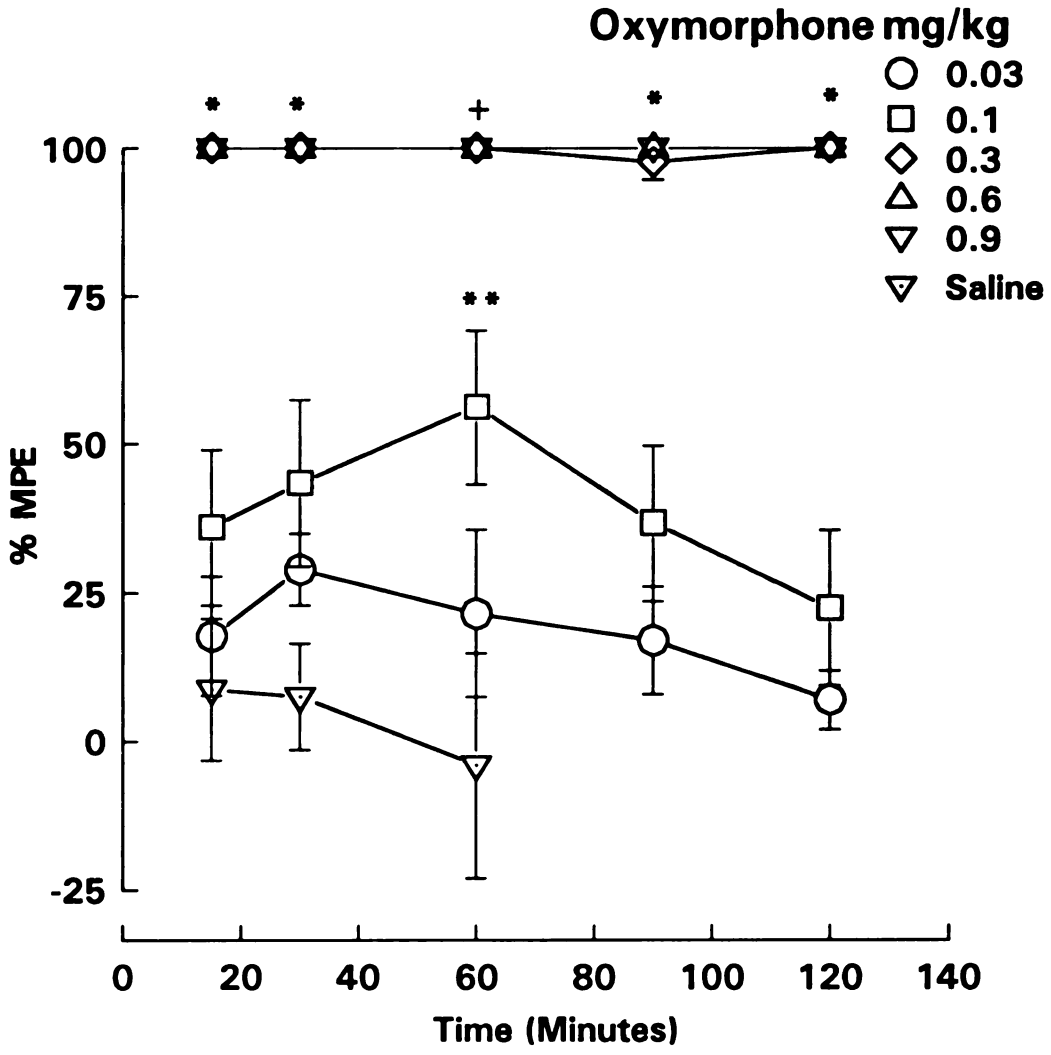

Figure 7. Colorectal balloon distension threshold after administration of oxymorphone.

*0.9, 0.6 and 0.3 mg/kg significantly different ($p < 0.05$) from all other dosages and saline. **0.1 mg/kg significantly different from 0.03 mg/kg and saline control. +0.9, 0.6 and 0.3 mg/kg significantly different from 0.03 mg/kg and saline. MPE = maximum possible effect.

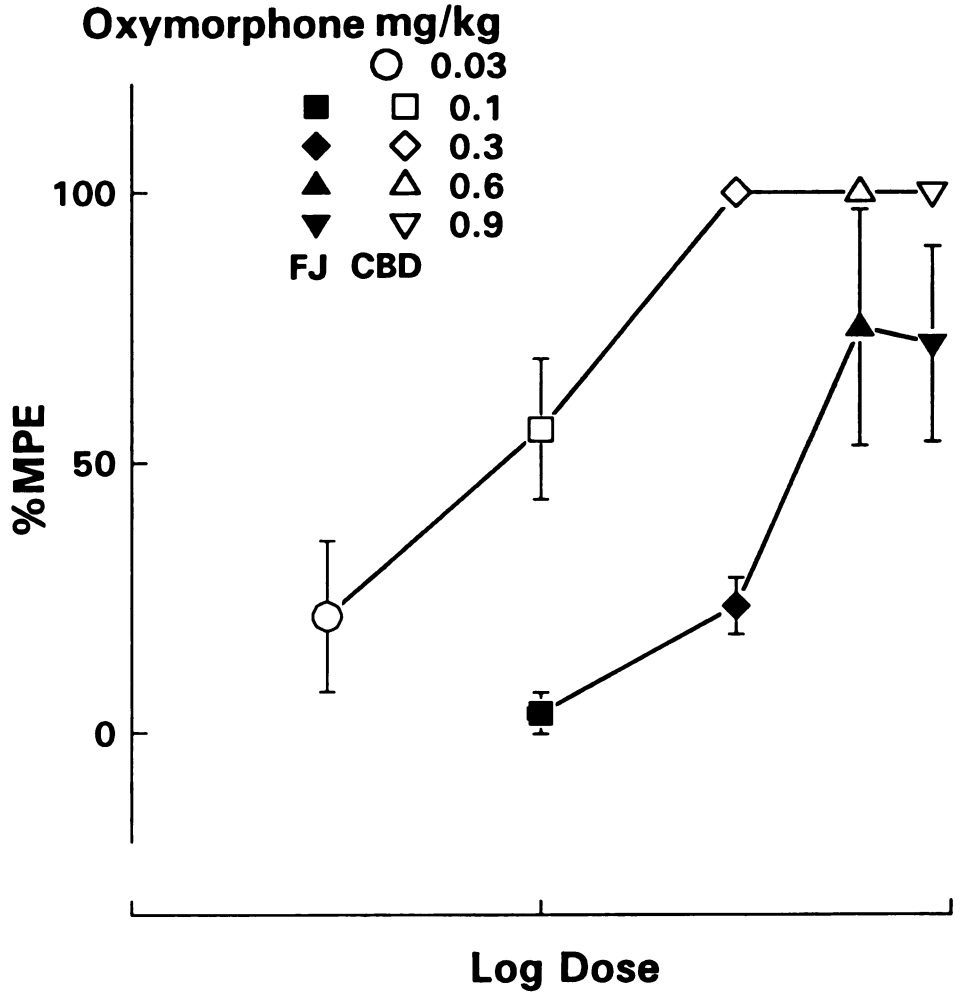

Figure 8. Oxymorphone dose-response comparing flinch-jump and colorectal balloon distension nociceptive thresholds 60 minutes post-drug administration.

MPE = maximum possible effect.

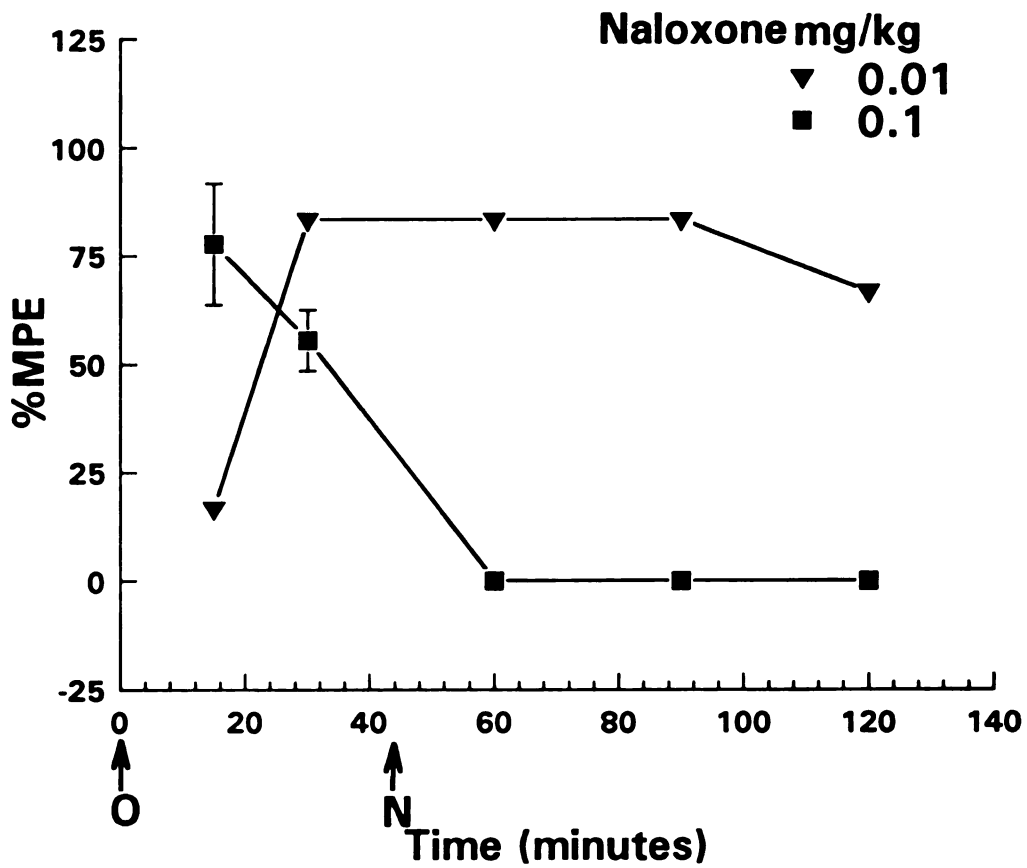

Figure 9. Colorectal balloon distension threshold after 0.05 mg/kg oxymorphone (O) and naloxone (N) treatment 45 minutes after oxymorphone.

MPE = maximum possible effect.

There were no significant increases in flinch-jump NT following administration of butorphanol (Figure 10). The CBD nociceptive threshold was increased following butorphanol. However, this effect had a relatively short duration. At 30 minutes, only the 0.1 mg/kg dosage significantly ($p < 0.05$) increased the NT above that for saline. Further, only 0.1 and 0.5 mg butorphanol/kg increased the NT significantly ($p < 0.05$) above that for saline at 60 minutes (Figure 11). The dose-response curves for butorphanol in both the FJ and CBD assays were evaluated at 30 minutes (Figure 12). Dose-response for butorphanol in the CBD procedure was typical of agonist/antagonist activity and demonstrated a ceiling effect at doses greater than 0.1 mg/kg. The CBD time course for NT following butorphanol (Figure 11) indicated that by 60 minutes, much of the antinociceptive effect of this drug was gone. Therefore, in the naloxone reversal studies a second dose of 0.1 mg butorphanol/kg was administered at 60 minutes to insure that equivocal results would not be obtained. Naloxone (0.4 mg/kg) significantly reversed the antinociceptive effect compared to saline (Figure 13). Several other doses of naloxone were given to determine the minimum dose necessary to reverse the antinociceptive effect (Figure 14). Naloxone doses as low as 0.05 mg/kg reduced the antinociceptive effect of 0.1 mg butorphanol/kg compared to saline in CBD (Figure 15).

There were no significant changes in NT after administration of ibuprofen (Figure 16). Following SQ ibuprofen approximately 50% of the rats developed ulcerations at the injection site. As a result, it was decided that it would be best to discontinue SQ ibuprofen injections and the drug was not evaluated further. Studies on non-steroidal anti-inflammatory (NSAID) agents were continued using Ketorolac tromethamine. There were no significant NT differences following 30 mg ketorolac/kg in FJ or CBD procedures compared to saline (Figures 17 & 18). Diazepam (1.0 mg/kg) was evaluated in both the FJ and CBD. There were no significant differences after diazepam administration in NT for FJ or CBD (Figures 19 and 20).

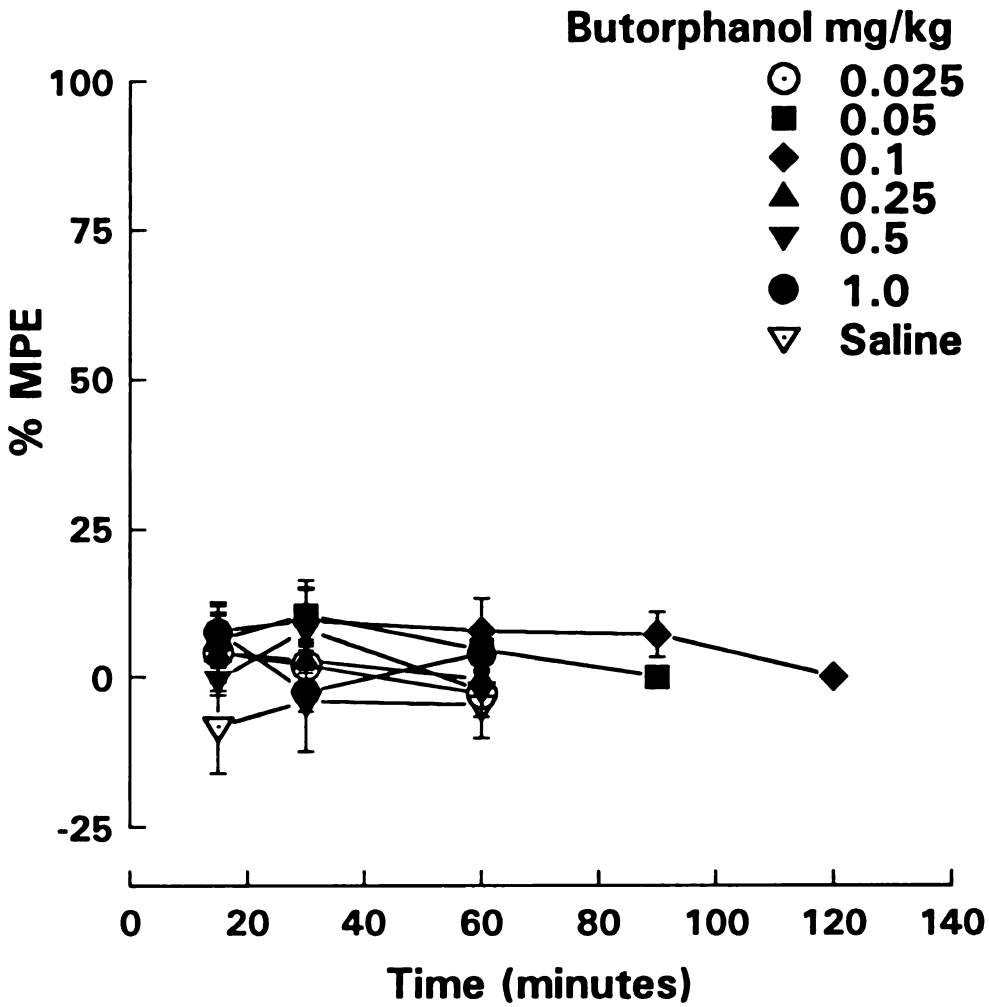

Figure 10. Flinch-jump thresholds following butorphanol administration.

MPE = maximum possible effect.

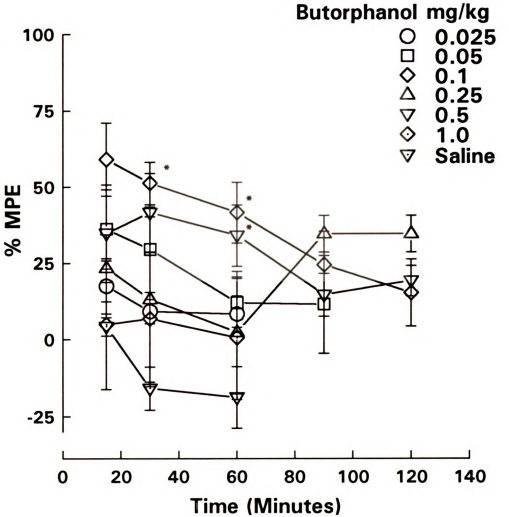

Figure 11. Colorectal balloon distension thresholds following butorphanol administration.

*significant difference ($p < 0.05$) from saline controls.
MPE = maximum possible effect.

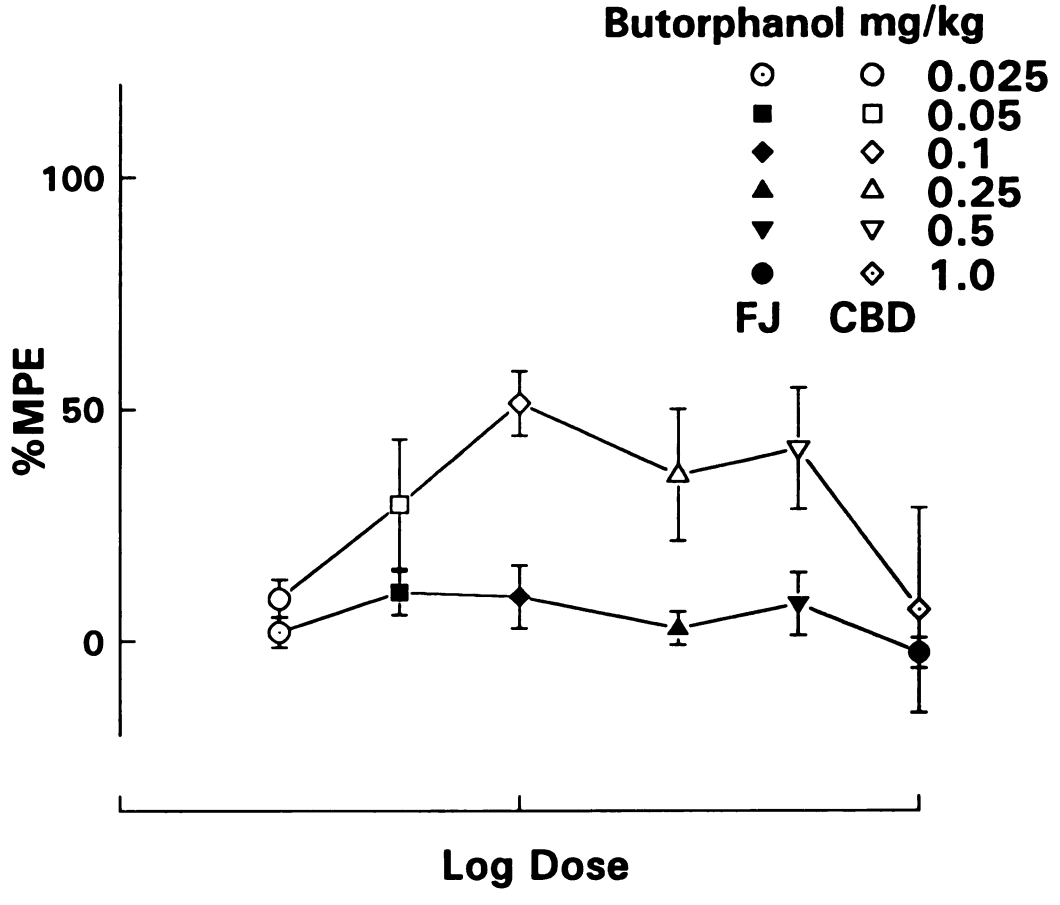

Figure 12. Butorphanol dose-response comparisons between flinch-jump and colorectal balloon distension thresholds at 30 minutes post-drug administration.

MPE = maximum possible effect.

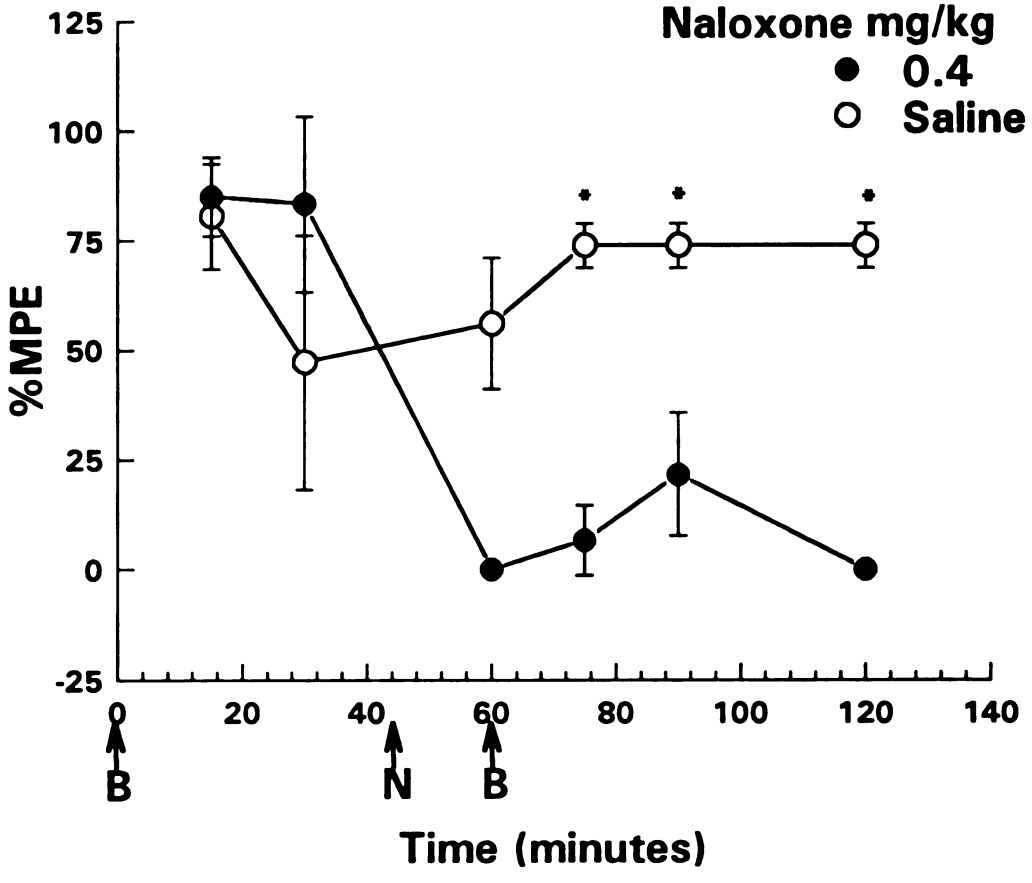

Figure 13. Colorectal balloon distension threshold after administration of 0.1 mg butorphanol/kg (B) and 0.4 mg naloxone/kg or equivalent volume saline (N).

*saline significantly greater ($p < 0.05$) CBD threshold than naloxone (0.4 mg/kg). MPE = maximum possible effect.

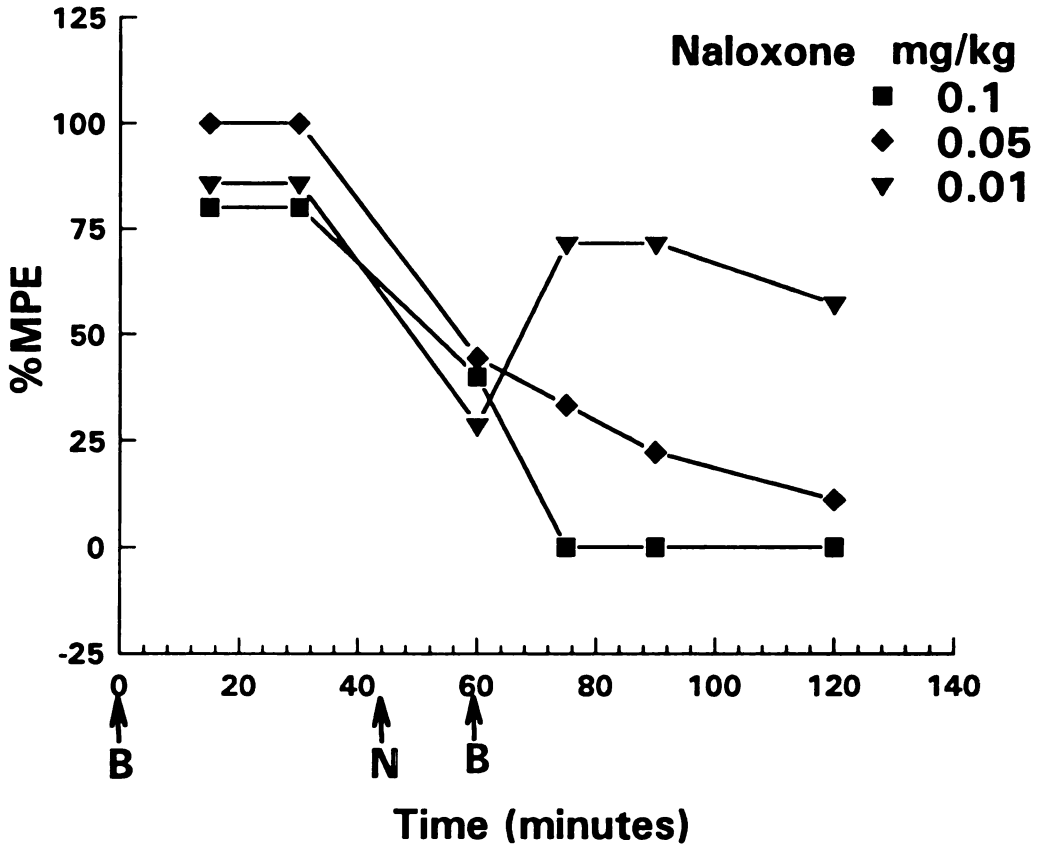

Figure 14. Colorectal balloon distension threshold after 0.1 mg butorphanol/kg (B) and naloxone doses(N).

MPE = maximum possible effect.

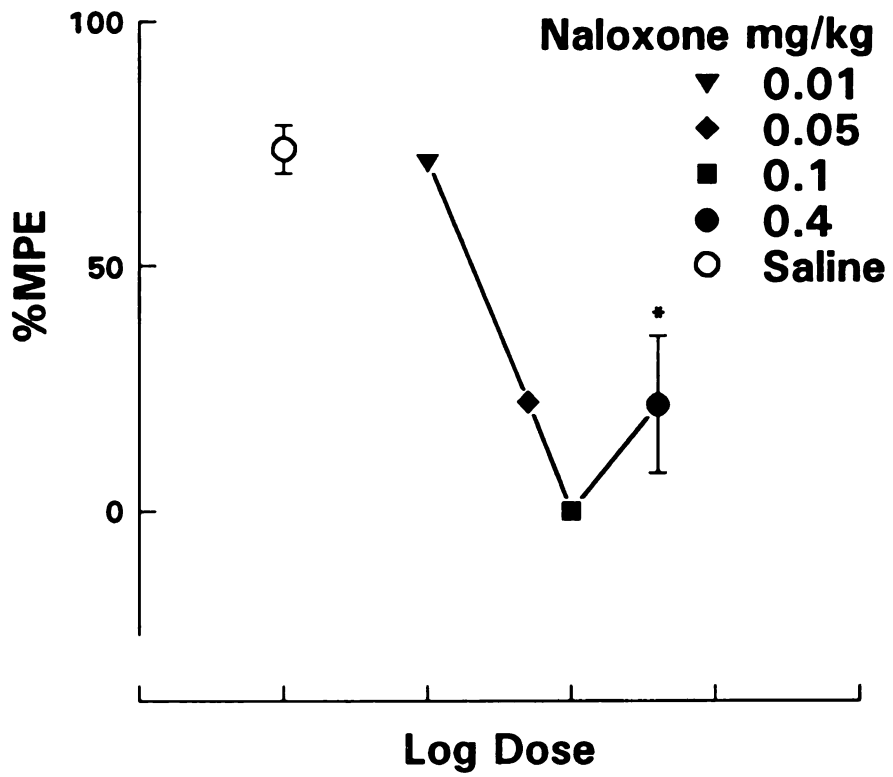

Figure 15. Dose-response of naloxone to reverse the antinociceptive effect of two doses of butorphanol (0.1 mg/kg each) at the 90 minute time point.

*naloxone significantly different ($p < 0.05$) from saline. n = 1 for naloxone 0.01, 0.05 and 0.1 mg/kg doses. MPE = maximum possible effect.

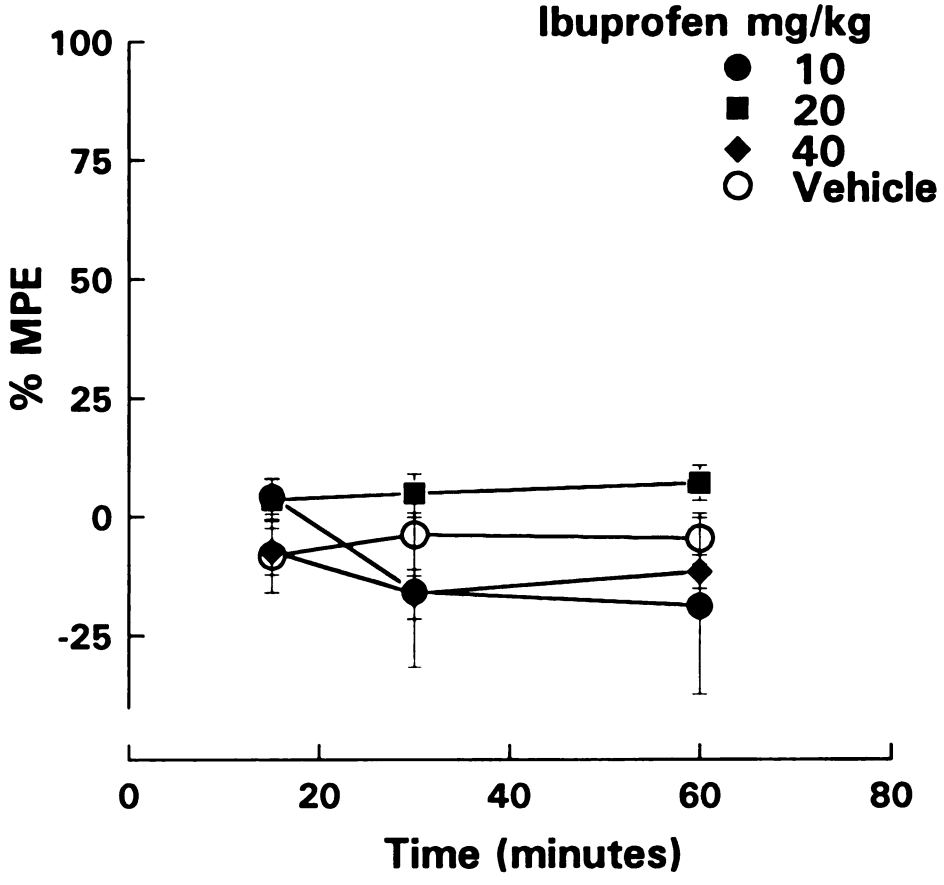

Figure 16. Flinch-jump threshold following ibuprofen. MPE = maximum possible effect.

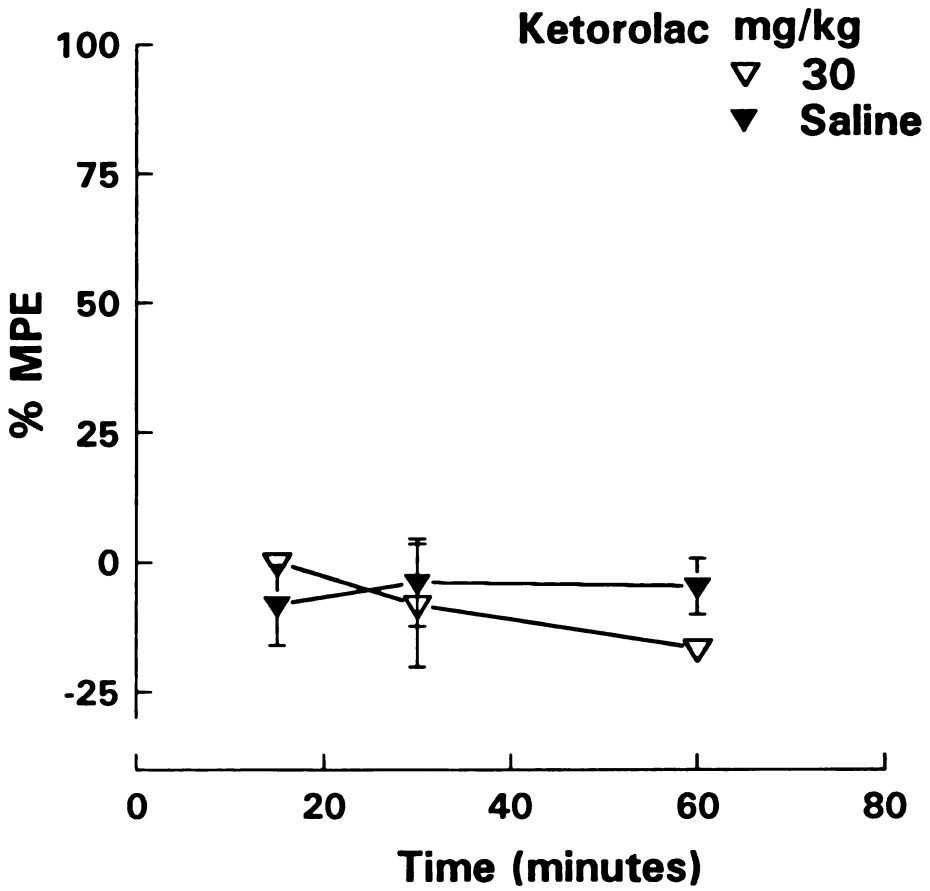

Figure 17. Flinch-jump threshold following ketorolac. MPE = maximum possible effect.

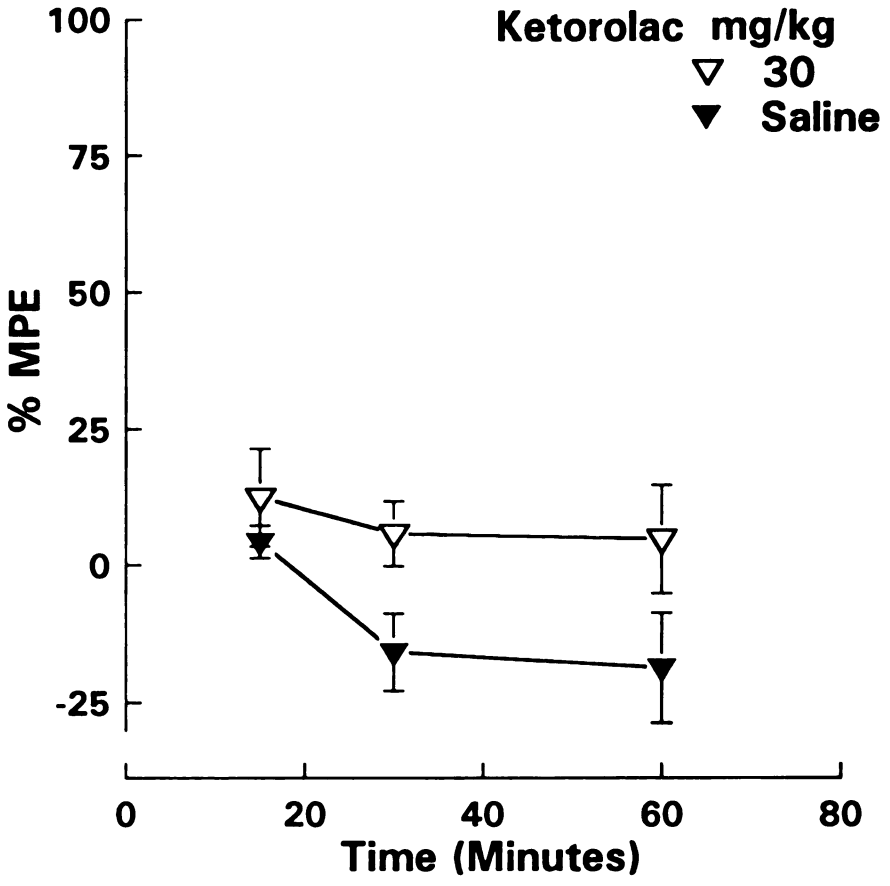

Figure 18. Colorectal balloon distension threshold following ketorolac.

MPE = maximum possible effect.

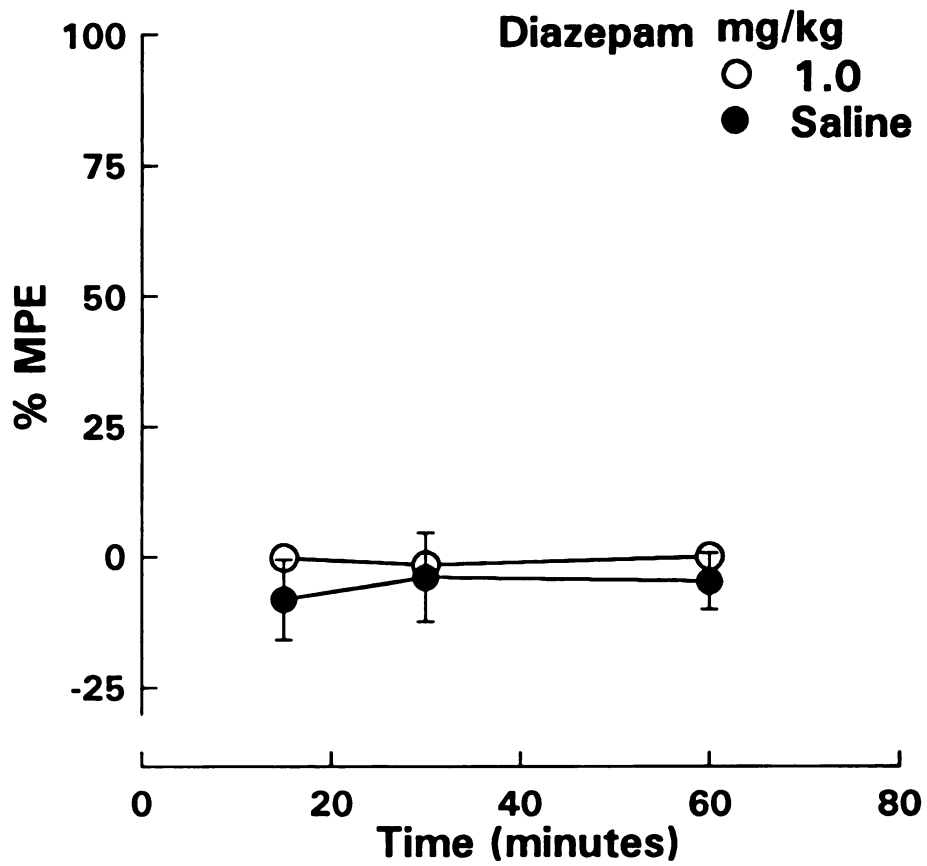

Figure 19. Flinch-jump threshold following diazepam. MPE = maximum possible effect.

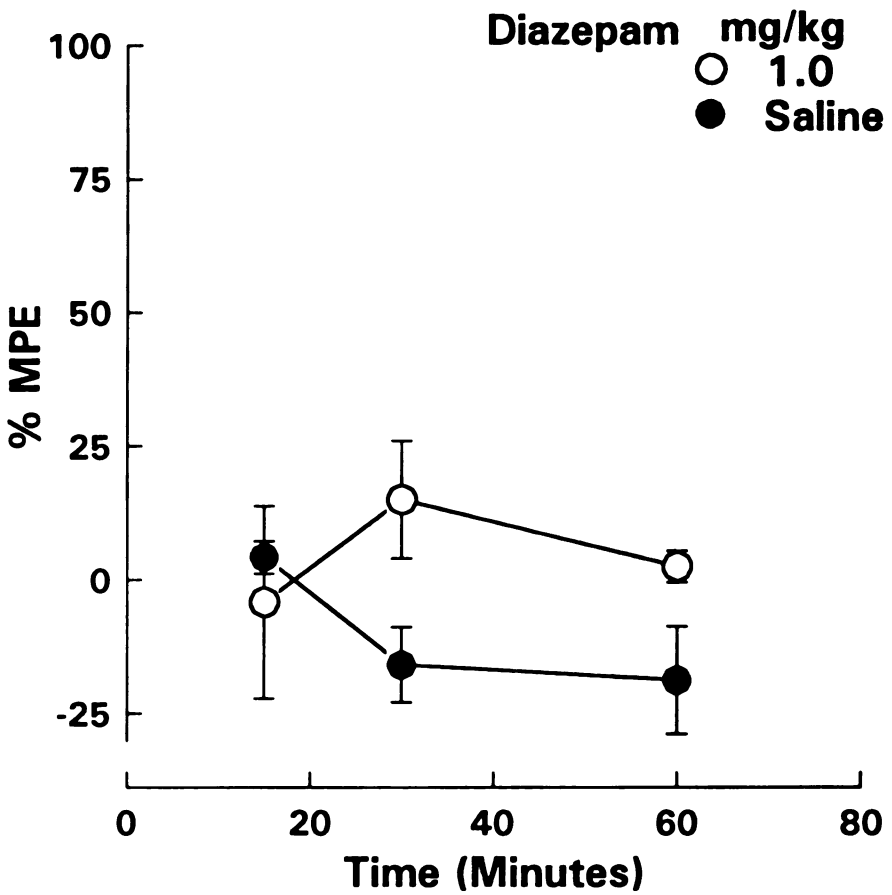

Figure 20. Colorectal balloon distension threshold following diazepam.

MPE = maximum possible effect.

DISCUSSION

It is well known that mu-opioid agonists are highly effective analgesics and often represent drugs of choice for management of moderate to severe pain. Yet, a complete mechanistic understanding of opioids remains elusive. However, brain sites related to analgesic effects have been mapped using intracerebral micro-injection of opioids (Yaksh and Rudy, 1978). Studies have confirmed that analgesia from systemic administration of mu opioids can be explained in part by the effects of these drugs in discrete groups of brain stem neurons (Gebhart, 1982). There is general agreement that modulation of nociceptive transmission is accomplished by a complex network of neurons in several brain regions, including the medullary periaqueductal grey (PAG) and rostral ventral medulla (RVM), and in the superficial dorsal horn of the spinal cord (Basbaum and Fields, 1984). The analgesic effects of systemic opioids are likely due to simultaneous effects at both supra-spinal and spinal sites in this network. This possibility is well supported by studies in which micro-injections of naloxone in the PAG, RVM, or spinal cord block analgesia obtained with systemic mu agonists (Jaquet and Lajtha, 1976; Azami et

al.,1982; Yaksh and Rudy, 1977). Additionally, ascending projections from PAG and RVM may influence the motivational/affective aspects of nociception. These midbrain regions send fibers to intralaminar thalamic and hypothalamic nuclei which project on to limbic structures. Casey (1980a) asserted that narcotic analgesics may act in the reticular formation to inhibit this rostrally projecting pathway known to play an important role in the motivational/affective aspects of nociception. This may account for observations whereby mu agonists reduce suffering without altering the ability to discriminate that stimuli are noxious.

Many of the standard assays for screening analgesic drugs use thermal or mechanical noxious stimuli applied to superficial somatic tissues. These procedures are easily employed in pharmaceutical settings to rapidly screen numerous compounds for analgesic activity. However, there is a major concern that these tests do not effectively model the most common clinical "pains" which are often more severe, often related to tissue damage, and induce a significant amount of suffering in human and veterinary patients. In this context how does the modified Evans FJ and CBD compare to the ideal nociceptive test discussed earlier, e.g. quantification, validity in terms of predicting clinical efficacy of analgesics, reliability/repeatability, simplicity, and sensitivity?

The FJ method was easy to use, and many determinations could be done rapidly. Furthermore, this test used a reliable behavioral response which did not change over time nor throughout many repeated trials, and induced no apparent tissue damage. These results demonstrated that the FJ test selected only the larger doses of oxymorphone as analgesic. One might postulate that such a result suggests that this mu agonist is selectively more effective than the other drugs tested. However, the oxymorphone ED_{50} (95% CL) 0.55 (0.23 - 1.34) mg/kg observed in the FJ procedure was significantly greater than antinociceptive doses (0.009 mg/kg) described in rats by other nociceptive tests (Steinfels and Cook, 1986), or the ED_{50} (95% CL) 0.07 (0.03 - 0.16)mg/kg observed in CBD. Cutaneous electrical stimulation likely excites more than just nociceptive systems and this may explain why the larger doses of oxymorphone were required to inhibit the FJ response. These findings for oxymorphone in the FJ procedure would support the assertion that analgesic doses of opioids do not disrupt discrimination. Only at the highest doses when prominent extrapyramidal side-effects were present, was it noted that the FJ threshold was increased. Since the dose required to increase the threshold in the FJ procedure was greater than that shown to be clinically effective as an analgesic, perhaps this procedure elicited a discriminatory response rather than a purely nociceptive response.

The CBD method shows great promise as a reliable means of screening for analgesic drug activity. Both classes of opioids (mu and kappa) increased the nociceptive threshold in rats. This test was quantifiable, CBD thresholds could be precisely measured repeatedly within the same day and from day to day. Pseudoaffective behavioral responses were also reliably measured and quantified. Previous experience has shown that careful control of the stimulus is necessary to prevent tissue damage to colon mucosa. The evidence reported here suggests that this method is selective and sensitive to the effects of opioid analgesics. Doses as low as 0.03 mg oxymorphone/kg demonstrate antinociceptive efficacy and the agonist/antagonist butorphanol was also effective to a lesser degree than was oxymorphone. These results support the CBD model as a valid method of screening drugs with antinociceptive potential.

The multiple actions of butorphanol at different receptors makes this a difficult drug to evaluate. The goal of this study was not to determine the mechanistic action of butorphanol, but rather to validate the CBD model by determining that doses similar to those used to manage pain would be selected by the CBD test. In this model, butorphanol had characteristics which resemble other agonist-antagonist analgesics. It exhibited a typical ceiling dose-response pattern due to antagonism of antinociceptive effects at higher doses. Even with

carefully selected doses, butorphanol was only a partial agonist; at doses above ED_{50} (0.1 mg butorphanol/kg) had no greater antinociceptive effect.

Besse et al., (1991) used radioligand binding studies to determine the relative densities of opioid receptors in the rat spinal cord. These data showed that of all opioid receptors 7 to 10% bind a kappa specific ligand, compared to 70 to 74% which bind a mu specific ligand. This characteristic of receptor distribution was cited as an explanation for differences in efficacy between kappa and mu opioid agonists. The low number of kappa opioid receptors in rat spinal cord may explain why the antinociceptive effects of butorphanol were highly variable. If effective antinociception requires selective kappa opioid binding, then drugs such as butorphanol must be carefully titrated to avoid side effects associated with mu or sigma opioid receptor binding. One side effect observed in these studies was increased struggling and agitation in the towel wrap restraint, even though CBD thresholds were elevated.

One unexpected result was observed in the naloxone reverseal of butorphanol antinociceptive effects. Due to mu receptor selectivity, it has been shown that naloxone reversal of kappa opioid related antinociception requires approximately four times the dose of naloxone (Millan et al., 1988). The studies reported herein, showed that both oxymorphone and butorphanol antinociception could be

reversed with naloxone at doses as low as 0.1 mg/kg (see Figures 8 and 15). Thus, it cannot be inferred that butorphanol antinociception in the rat is exclusively dependent on kappa opioid receptor binding. Mu receptor binding may be involved in the antinociceptive effects observed for butorphanol. However, these data are somewhat preliminary; A full pA2 analysis was beyond the scope of this study. Additionally, a satisfactory answer to the question of exclusive kappa opioid involvement in rat antinociception would require the use of more selective kappa opioid receptor agonists and antagonists.

Neither the non-steroidal anti-inflammatory analgesic drugs (NSAIDs) nor the non-analgesic drugs tested were effective in increasing the CBD nociceptive threshold or FJ threshold. CBD has been shown to stimulate primary nociceptive afferent fibers and thus probably does not acutely involve prostaglandin synthesis. This does not rule out the possibility that SQ administration of NSAIDs may not achieve sufficient blood levels to reach the antinociceptive therapeutic range.

Summary

Evidence presented here suggests that the modified FJ procedure does not represent a purely nociceptive model. Cutaneous electrical stimulation is not a natural nociceptive stimulus and probably excites more than just nociceptive systems. It was apparent in these studies that the modified FJ procedure may preferentially stimulate tactile discriminatory pathways. Only the highest oxymorphone doses altered the FJ threshold. Conversely, the CBD procedure may preferentially stimulate nociceptive specific pathways. Dosages of oxymorphone and butorphanol with known clinical efficacy were able to increase CBD thresholds in a dose-dependent fashion, which could be reversed by naloxone. Additionally, the CBD demonstrated opioid selectivity since neither NSAIDs nor diazepam were selected by this method as antinociceptive. Taken together, these studies call into question the validity of the modified FJ procedure, while supporting CBD as a valid model for screening drugs with antinociceptive potential.

BIBLIOGRAPHY

BIBLIOGRAPHY

Azami, J., M. B. Llewelyn, and M. H. T. Roberts (1982). The contribution of nucleus reticularis paragigantocellularis and nucleus raphe magnus to the analgesia produced by systemically administered morphine, investigated with the microinjection technique. Pain. 12, 229-246.

Barber, R. P., J. E. Vaughn, K. Saito, B. J. McLaughlin, and E. Roberts (1978). GABAergic terminals are presynaptic to primary afferent terminals in the substantia gelatinosa of the rat spinal cord. Brain Research. 141, 35-55.

Basbaum, A. I., (1984). Anatomical substrates of pain and pain modulation and their relationship to analgesic drugs. In: Analgesics: Neurochemical, Behavioral and Clinical Perspective. M. Kuhar and C. Pasternak (eds). Raven Press, New York, pp. 97-123.

Basbaum, A. I., and H. L. Fields (1984). Endogenous pain control systems: Brainstem spinal pathways and endorphin circuitry. Annual Reviews in Neuroscience. 7, 309-338.

Beecher, H. K., (1957). The measurement of pain. Prototype for the quantitative study of subjective responses. Pharmacological Reviews. 9, 59-209.

Besse, D., M. C. Lombard, and J. M. Besson (1991). Autoradiographic distribution of μ, δ and κ opioid binding sites in the superficial dorsal horn, over the rostrocaudal axis of the rat spinal cord. Brain Research. 548, 287-291.

Bonica, J. J., (1990). Anatomic and physiologic basis of nociception and pain. In: The Management of Pain. Vol. I. J. J. Bonica (ed). Lea & Febiger, Philadelphia, London, pp. 28-94.

Bowsher, D. (1976). Role of the reticular formation in response to noxious stimulation. Pain. 2, 361-378.

Brown, A. G., (1981). The organization of the dorsal horn. In: Organization in the Spinal cord. The anatomy and physiology of identified neurons. Springer-Verlag, Berlin. pp. 136-153

Casey, K. L., (1966). Unit analysis of nociceptive mechanisms in the thalamus of the awake squirrel monkey. Journal of Neurophysiology. 29, 727-750.

Casey, K. L., (1971). Somatosensory responses of bulboreticular units in awake cat: Relation to escape producing stimuli. Science. 173, 77-80.

Casey, K. L., (1980a). Reticular formation and pain: Toward a unifying concept. In Pain. J. J. Bonica (ed). Raven Press, New York. pp. 93-105.

Casey, K. L., (1980b). Supraspinal mechanisms and pain: The reticular formation. In Pain and Society. H. W. Kosterlitz and L. Y. Terenius (eds). Verlag Weinheim, Chemie. pp. 183-200.

Cervero F., A. Iggo, and H. Ogawa (1976). Nociceptor driven dorsal horn neurons in the lumbar spinal cord of the cat. Pain. 2, 5-24.

Cerevero, F., (1983). Mechanisms of visceral pain. In: Persistent Pain. Vol. 4. Grune and Stratton, New York.

Cerevero, F. and J. F. B. Morrison (1986).Visceral Sensation. Elsevier, Amsterdam, New York, Oxford.

Chavkin, D., I. F. James, and A. Goldstein (1982). Dynorphin is a specific endogenous ligand of the Kappa opioid receptor. Science. 215, 413-415.

Colburn, R.W., D. W. Coombs, C. C. Degnan and L. L. Rogers (1989). Mechanical visceral pain model: chronic intermittent intestinal distention in the rat. Physiology and Behavior. 45, 191-197.

Dennis, S. G., M. Choinière, and R. Melzack (1980). Stimulation-produced analgesia in rats: Assessment by two pain tests and correlation with self-stimulation. Experimental Neurology. 68, 295-309.

Desarmenien, M., P. Feltz, and P. M. Headley (1980). Does glial uptake affect GABA responses? An intracellular study on rat dorsal root ganglion neurones in vitro. Journal of Physiology (London). 307, 163-182.

Dong, W. K., and I. H. Wagman (1976). Modulation of nociceptive responses in the thalamic posterior group of nuclei. In: Advances in Pain Research and Therapy. Vol. 1. J. J. Bonica and D. Albe-Fessard (eds). Raven Press, New York. pp. 455-460.

Dubner, R., D. D. Price, R. E. Beitel and J. W. Hu (1977). Peripheral neural correlates of behavior in monkey and human related to sensory-discriminative aspects of pain. In: Pain in the Trigeminal Region. D. J. Anderson and B. Matthews (eds). Elsevier, North Holland. pp. 57-66.

Dubner, R., and G. J. Bennett (1983). Spinal and trigeminal mechanisms of nociception. Annual Reviews in Neuroscience. 6, 381-418.

Dubner, R. (1985). Specialization in nociceptive pathways: Sensory discrimination, sensory modulation, and neural connectivity. In Advances in Pain Research and Therapy, Vol. 9, H. L. Fields, R. Dubner and F. Cervero (eds). Raven Press, New York, pp. 111-137.

Durham, R. A., D. C. Sawyer, and R. H. Rech (1992). A visceral pain model to evaluate augmentation of butorphanol analgesia when combined with xylazine in cats. (To be published).

Evans W. O., (1961). A new technique for the investigation of some analgesic drugs on a reflexive behavior in the rat. Psychopharmacologica. 2, 318-325.

Evans, W. O. (1962). A comparison of the analgetic potency of some analgesics as measured by the flinch jump procedure. Psychopharmacologica. 3, 51-54.

Evans, W. O., (1964). A critical review of some new methods in animal analgesiometry. Journal of New Drugs. 4, 179-189.

Fields, H. L., P. C. Emson, B. K. Leigh, R. F. T. Gilbert and L.L. Iversen (1980). Multiple opiate receptor sites on primary afferent fibers. Nature (London) 284, 351-353.

Gebhart, G. F. (1982). Opiate and opioid peptide effects on brain stem neurons: Relevance to nociception and antinociceptive mechanisms. Pain. 12, 93-140.

Gebhart, G. F., and T. J. Ness, (1991). Central mechanisms of visceral pain. Canadian Journal of Physiology and Pharmacology. 69, 627-634.

Giesler, G. J., G. Urca, J. T. Cannon and J. C. Liebeskind (1979). Response properties of neurons of the lateral cervical nucleus in the rat. Journal of Comparative Neurology. 186, 65-78.

Giesler, G. J., H. R. Spiel and W. D. Willis (1981a). Organization of spinothalamic tract axons within the rat spinal cord. Journal of Comparative Neurology 195, 243-252.

Giesler, G. J., R. P. Yezierski, K. D. Gerhart and W. D. Willis (1981b). Spinothalamic tract neurons that project to medial and/or lateral thalamic nuclei: evidence for a physiologically novel population of spinal cord neurons. Journal of Neurophysiology. 46, 1285-1308.

Gobel, S., (1978). Golgi studies of the neurons in layer II of the dorsal horn of the medulla (trigeminal nucleus caudalis). Journal of Comparative Neurology. 180, 395-314.

Goligher, J. C., and E. S. R. Hughes (1951). Sensibility of the rectum and colon: its role in the mechanism of anal continence. Lancet. i-ii, 543-548.

Harper, A. A., and S. N. Lawson (1985). Conduction velocity is related to morphological cell type in rat dorsal root ganglion neurones. Journal of Comparative Neurology. 237, 195-215.

Harris, L. S. and A. K. Pierson (1964). Some narcotic antagonists in the benzomorphan series. Journal of Pharmacology and Experimental Therapeutics. 143, 141-148.

Hoheisel, V., and S. Mense (1986). Non-myelinated afferent fibers do not originate exclusively from the smallest dorsal root ganglion cells in the cat. Neuroscience Letters. 72, 153-157.

Houghton, K. J., R. H. Rech, D. C. Sawyer, R. A. Durham, T. Adams, M. A. Langham, and E. L. Striler (1991). Dose-response of intravenous butorphanol to increase visceral nociceptive threshold in dogs. Proceedings of the Society for Experimental Biology and Medicine. 197(3), 290-296.

Hylden J. L. K., H. Hayashi, G. J. Bennett and R. Dubner (1985). Spinal lamina I neurons projecting to the parabrachial area to the cat midbrain. Brain Research 336, 195-198.

Hylden J. L. K., H. Hayashi, R. Dubner and G. J. Bennett (1986a). Physiology and morphology of the lamina I spinomesencephalic projection. Journal of Comparative Neurology. 247, 505-515.

Hylden J. L. K., H. Hayashi and G. J. Bennett (1986b). Lamina I spinomesencephalic neurons in the cat ascend via the dorsolateral funiculi. Somatosensory Research. 4, 31-41.

Iggo, A., (1974). Pain receptors. In: Recent Advances in Pain: Pathophysiology and Clinical Aspects. J. J. Bonica, P. Procacci and C. A. Pagni (eds). pp. 3-35. Charles C. Thomas, Springfield, IL.

Jacquet, Y. F., and A. Lajtha (1976). The periaqueductal gray: Site of morphine analgesia and tolerance as shown by 2-way cross tolerance between systemic and intracerebral injections. Brain Research. 103, 501-513.

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. In: Visceral Sensation, Progress in Brain Research. Vol. 67, F. Cervero and J. F. B. Morrison (eds). Elsevier, Amsterdam, Oxford, New York, pp. 87-114.

Jones, E. G., and D. P. Friedman (1982). Projection pattern of functional components of the thalamic ventrobasal complex in monkey somatosensory cortex. Journal of Neurophysiology. 48, 521-544.

Jones, M. W., C. J. Hodge, A. V. Apkarian and R. T. Stevens (1985). A dorsolateral spinothalamic pathway in cat. Brain Research. 335, 188-193.

Kerr, F. W. L., and H. H. Lippman (1974). The primate spinothalamic tract as demonstrated by anterolateral cordotomy and commissural myelotomy. In: Advances in Neurology. Vol. 4. J.J. Bonica (ed). Raven Press, New York. pp. 147-156.

Keveter, G. A. and W. D. Willis (1982). Spinothalamic cells in the rat lumbar cord with collaterals to the medullary reticular formation. Brain Research. 238, 181-185.

Kissel, J. W., J. R. Albert, and G. C. Boxhill (1961). The pharmacology of prodilidine hydrochloride, a new analgetic agent. Journal of Pharmacology and Experimental Therapeutics. 134, 332-340.

LaMotte, R. H. and N. J. Campbell (1978). Comparison of responses of warm and nociceptive C-fiber afferents in monkey with human judgements of thermal pain. Journal of Neurophysiology. 41, 509-528.

Lawson, S. N. (1979). The postnatal development of large light and small dark neurons in mouse dorsal root ganglia: a statistical analysis of cell numbers and size. Journal of Neurocytology. 8, 275-294.

Lawson, S. N., A. A. Harper, E. I. Harper, J. A. Sarson and B. H. Anderton (1984). A monoclonal antibody against neurofilament protein specifically labels a subpopulation of rat sensory neurones. Journal of Comparative Neurology. 228, 263-272.

Leah J. D., A. A. Cameron, and P. J. Snow (1985). Neuropeptides in physiologically identified mammalian sensory neurones. Neuroscience Letters. 56, 257-263.

Leek, B. F., (1972). Abdominal visceral receptors. In: Handbook of Sensory Physiology. Vol. 3/1. E. Neil (ed). pp. 113-160. Springer-Verlag, Berlin.

Lewis, T. (1942), Pain. Macmillan, London. pp. 1-192.

Lineberry, C. G., (1981). Laboratory animals in pain research. In Methods of Animal Experimentation. Vol. VI. W. I. Gay (ed). Academic Press, New York. pp. 237-311.

Lowe, J. E., (1969). Pentazocine (Talwin-V) for the relief of abdominal pain in ponies-a comparative evaluation with description of a colic model for analgesia evaluation. Proceedings of the American Association of Equine Practitioners. 11, 31-46.

Lowe, J. E., H. F. Hintz, and H. F. Schryver (1970). A new technique for long-term cecal fistulation in ponies. American Journal of Veterinary Research. 31, 1109-1111.

MacDonald R. L., and M. A. Werz (1986). Dynorphin A decreases voltage-dependent calcium conductance of mouse dorsal root ganglion neurons. Journal of Physiology (London) 377, 237-249.

Mehler, W. R. (1969). Some neurological species differences: a posteriori. Annals of the New York Academy of Science, 167, 424-468.

Merskey, H. (1979). Pain terms: a list with definitions and notes of usage. Recommended by the IASP Subcommittee on Taxonomy. Pain 6, 249-252.

Miletic, V., M. J. Hoffert, M. A. Ruda, R. Dubner, and Y. Shigenaga (1984). Serotonergic axonal contacts on identified cat spinal dorsal horn neurons and there correlation with nucleus raphe magnus stimulation. Journal of Comparative Neurology 228, 129-141.

Millan, M., (1989). Kappa-opioid receptor-mediated antinociception in the rat. I. Comparative actions of mu- and kappa-opiods against noxious thermal, pressure and electrical stimuli. Journal of Pharmacology and Experimental Therapeutics. 251, 334-341.

Morrison, J. F. B., (1977). The afferent innervation of the gastrointestinal tract. In Nerves and the Gut. F. P. Brooks and P. W. Evers (eds). C. B. Slack, Thorofare, NJ. pp. 297-322.

Mudge, A. W., S. E. Leeman and G. D. Fischbach (1979). Enkephalin inhibits release of substance P from sensory neurons in culture and decreases action potential duration. Proceedings of the National Academy of Science. 76, 526-530.

Nashold B.S., W. P. Wilson and D.G. Slaughter (1969). Sensations evoked by stimulation in the midbrain of man. Journal of Neurosurgery. 30, 14-24.

Ness, T. J. and G. F. Gebhart (1988). Colorectal distension as a noxious visceral stimulus: physiologic and pharmacologic characterization of pseudaffective reflexes in the rat. Brain Research. 450, 153-169.

Netter, F. H. (1983). Nervous System Anatomy and Physiology. Vol. 1. CIBA Collection of Medical Illustrations. CIBA, West Caldwell, NJ.

Noordenbos, W, and P. D. Wall (1976). Diverse sensory functions with an almost totally divided spinal cord. A case of spinal cord transection with preservation of part of one anterolateral quadrant. Pain. 2, 185-195.

Olds, M. E., and J. Olds (1963). Approach-avoidance analysis of rat diencephalon. Journal of Comparative Neurology. 120, 259-295.

Otsuka, M., S. Konishi, M. Yanagisawa, A. Tsunoo and H. Akagi (1982). Role of substance P as a sensory transmitter in spinal cord and sympathetic ganglia. In: Substance P in the Nervous System. Ciba Foundation symposium, vol 16. Pitman, London, pp. 13-34.

Patrick, J. T., W. J. McBride, and D. L. Felten (1983). Distribution of glycine, GABA, aspartate and glutamate in the rat spinal cord. Brain Research. 10, 415-418.

Peschanske, M., P. W. Mantyh and J. M. Besson (1983). Spinal afferents to the ventrobasal thalamic complex in the rat: an anatomical study using wheat-germ agglutinin conjugated to horseradish peroxidase. Brain Research. 278, 240-244.

Peschanski, M. V. Kayser and J. M. Besson (1986). Behavioral evidence for a crossed ascending pathway for pain transmission in the anterolateral quadrant of the rat spinal cord. Brain Research. 376, 164-168.

Pippi, N. L., and W. V. Lumb (1979). Objective tests of analgesic drugs in ponies. American Journal of Veterinary Research. 40, 1082-1086.

Poggio, G. F., and V. B. Mountcastle, (1960). A study of the functional contributions of the leminiscal and spinothalamic systems to somatic sensibility. Bulletin of the Johns Hopkins Hospital. 106, 266-316.

Pomeranz B., P. D. Wall and W. V. Weber (1968). Cord cells responding to fine myelinated afferents from viscera, muscle and skin. Journal of Physiology. 199, 511-532.

Raffe, M. R., (1992). Animal models for the evaluation of analgesic agents. In: Animal Pain. C. E. Short and A. Van Poznak (eds). Churchill Livingstone, New York, NY. pp. 453-458.

Rech, R. H., D. C. Sawyer, and D. H. MacKenzie-Taylor (1987). Kappa agonist analgesia studied in several species with a measure of visceral pain. Proceedings of the 26th Annual Meeting of the American College of Neuropsychopharmacology.(Abstract).

Rexed, B., (1954). A cytoarchitectonic atlas of the spinal cord in the cat. Journal of Comparative Neurology. 100, 297-379.

Ruda, M. A., J. Coffield, and H. W. M. Steinbusch (1982). Immunocytochemical analysis of serotonergic axons in laminae I and II of the lumbar spinal cord of the cat. Journal of Neuroscience. 2, 1660-1671.

Sawyer D. C. and R. H. Rech (1987). Analgesia and behavioral effects of butorphanol, nalbuphine, and pentazocine in the cat. Journal of the American Animal Hospital Association. 23, 438-446.

Sawyer, D. C., R. H. Rech, and R. A. Durham (1990). Effects of ketamine and combination with acetylpromazine, diazepam, or butorphanol on visceral nociception in the cat. In: Status of Ketamine in Anesthesiology. NPP Books, Ann Arbor, MI.

Sawyer, D. C., R. H. Rech, R. A. Durham, T. Adams, M. A. Richter, and E. L. Striler (1991). Dose-response of butorphanol administered subcutaneously to increase visceral nociceptive threshold in dogs. American Journal of Veterinary Research. 52(11), 1826-1830.

Sawyer, D. C., R. H. Rech, T. Adams, R. A. Durham, M. A. Richter, and E. L Striler (1992a). Analgesia and behavioral responses of oxymorphone-acepromazine and meperidine-acepromazine following methoxyflurane and halothane anesthesia in the dog. American Journal of Veterinary Research. 53(8), 1361-1368.

Sawyer, D. C., R. H. Rech, and R. A. Durham (1992b). Does ketamine provide adequate visceral analgesia when used alone or in combination with acepromazine, diazepam or butorphanol in cats? Journal of the American Animal Hospital Association. (In Press).

Seybold , V. S. and R. P. Elde (1982). Neurotensin immunoreactivity in the superficial laminae of the dorsal horn of the rat: I. Light microscopic studies of cell bodies and proximal dendrites. Journal of Comparative Neurology. 205, 89-100.

Stanzione, P. and W. Zieglgansberger (1983). Action of neurotensin on spinal cord neurons in the rat. Brain Research. 268, 111-118.

Steinfels, G. F., and L. Cook (1986). Antinociceptive profiles of mu and kappa opioid agonists in a rat tooth pulp stimulation procedure. Journal of Pharmacology and Experimental Therapeutics. 236, 111-117.

Swett J. E., S. B. McMahon, P. D. Wall (1985). Long ascending projections to the midbrain from cells of lamina I and nucleus of the dorsolateral funiculus of the rat spinal cord. Journal of Comparative Neurology. 238, 401-416.

Taylor, D.C.M. and F.K. Pierau (1991). Nociceptive afferent Neurones. Studies in neuroscience No. 14, W. Winslow (Ed.) Manchester University Press, Manchester, New York.

Tilson, H. A., R. H. Rech and S. Stolman (1973). Hyperalgesia during withdrawal as a means of measuring the degree of dependence in morphine-dependent rats. Psychopharmacologia. 28, 287-300.

Wall, P. D. (1989). The dorsal horn. In: Textbook of Pain. 2nd. ed. P. D. Wall and R. Melzack (eds). Churchill Livingstone, New York pp. 102-111.

Werz M. A., and R. L. MacDonald (1982a) Opioid peptides decrease calcium dependent action potential duration of mouse dorsal root ganglion neurons in cell culture. Brain Research. 239, 315-321.

Werz M. A., and R. L. MacDonald (1982b). Heterogenous sensitivity of cultured dorsal root ganglion neurons to opioid peptides selective for mu and delta opiate receptors. Nature (London). 290, 730-733.

Werz M. A., and R. L. MacDonald (1983). Opioid peptides selective for μ- and δ-opiate receptors reduce calcium-dependent action potential duration by increasing potassium conductance. Neuroscience Letters. 42, 173-178.

White, J. C. and W. H. Sweet (1969). Pain and the Neurosurgeon. Thomas, Springfield, IL.

Willcockson, W. S., J. M. Chung, Y. Hori, K. H. Lee, and W. D. Willis (1984). Effects of iontophoretically released amino acids and amines on primate spinothalamic tract cells. Journal of Neuroscience. 4, 732-740.

Willis, W. D. and R. E. Coggeshall (1978), Sensory Mechanisms of the Spinal Cord. Plenum Press, New York.

Willis, W. D., D. R. Kenshalo Jr. and R. B. Leonard (1979). The cells of origin of the primate spinothalamic tract. Journal of Comparative Neurology. 188, 543-574.

Willis, W.D., (1982). Control of nociceptive transmission in the spinal cord. In: Progress in sensory physiology. vol. 3. H. Autrum, D. Ottson, E. Perl and R. F. Schmidt (eds). Springer-Verlag, New York.

Willis, W. D., (1985). The Pain System: The neural Basis of Nociceptive Transmission in The Mammalian Nervous System. In: Pain and Headache. Vol. 8. P. L. Gildenberg (ed). Karger, Basel. pp. 1-346.

Wood, P. L., (1984). Animal models in analgesic testing. In: Analgesics: Neurochemical, Behavioral, and Clinical Perspectives. Raven Press, New York, NY. p. 175.

Yaksh, T. L., and T. A. Rudy (1977). Studies on the direct spinal action of narcotics in the production of analgesia in the rat. Journal of Pharmacology and Experimental Therapeutics. 202, 411-428.

Yaksh, T. L., and T. A. Rudy (1978). Narcotic analgesics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. Pain. 4, 299-359.

Yaksh T. L., T. M. Jessell, R. Gamse, A. W. Mudge and S. E. Leeman (1980). Intrathecal morphine inhibits substance P release from mammalian spinal cord in vivo. Nature (London). 286, 155-57.

Yaksh, T. L., and D. L. Hammond (1982). Peripheral and central substrates in the rostral transmission of nociceptive information. Pain. 13, 1-85.

Yoshimura M., and R. A. North (1983). Substantia gelatinosa neurones hyperpolarized in vitro by enkephalin. Nature (London). 305, 529-530.

Zotterman, Y., (1939). Touch, pain and tickling: An electrophysiological investigation on cutaneous sensory nerves. Journal of Physiology (London). 95, 1-28.

GENERAL REFERENCES

Borchard, R. E., C. D. Barnes, and L. G. Eltherington (1990). Drug Dosage In Laboratory Animals A Handbook. 3rd. ed. Telford Press, Caldwell, NJ.

Brown, A. G., (1981). Organization in the Spinal cord. The anatomy and physiology of identified neurons. Springer-Verlag, Berlin.

Cervero, F. and A. Iggo (1980), The substantia gelatinosa of the spinal cord - a critical review. Brain. 103, 717-772.

Fields, H. L., R. Dubner, and F. Cervero (1985). Advances in Pain Research and Therapy Vol. 9: Proceedings of the Fourth World Congress on Pain. Raven Press, New York, NY.

Jänig, W. (1988) Pathophysiology of nerve following mechanical injury. In: Proceedings of the Vth World Congress on Pain, R. Dubner, G. F. Gebhart and M. R. Bond (eds). Elsevier, Amsterdam, Oxford, New York.

Kandel E. R., J. H. Schwartz, and T. M. Jessell (1991). Principles of Neuroscience. 3rd. ed. Elsevier, New York, NY.

Kerr, F. W. L. (1975), Neuroanatomical substrates of nociception in the spinal cord. Pain. 1, 325-356.

Kitchell R. and H. H. Erickson (1983). Animal Pain Perception and Alleviation. Waverly Press, Baltimore, MD.

Melzack, R., and P. D. Wall (1983). The Challenge of Pain. Basic Books, New York, NY.

Ness, T. J. and G. F. Gebhart (1990). Visceral pain: a review of experimental studies. Pain. 41, 167-234.

Ralston, H. R., (1984). Synaptic organization of the spinothalamic tract projection to the thalamus with special reference to pain. In Advances in Pain Research and Therapy. Vol. 6. L. Kruger and J. C. Liebeskind (eds). Raven Press, New York, NY. pp. 183-195.

Ruda, M. A., (1985). The pattern and place of nociceptive modulation in the dorsal horn: A discussion of the anatomically characterized neural circuitry of enkephalin, serotonin, and substance P. In Spinal Afferent Processing. T. L. Yaksh (ed) Plenum Press, New York, and London, pp. 141-164.

Short, C. E., and A. Van Poznak (1992). Animal Pain. Churchill Livingstone, New York, NY.

Wall, P. D., and R. Melzack (1989). Text Book of Pain. 2nd ed. Chruchill Livingstone New York, NY.

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