FACILITATORY INTERACTION BETWEEN PHARMACOLOGICALLY DISTINCTIVE GANGLIONIC CHOLINOCEPTIVE SITES

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

DAVID WELLS SNYDER

1969

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ABSTRACT

FACILITATORY INTERACTION BETWEEN
PHARMACOLOGICALLY DISTINCTIVE
GANGLIONIC CHOLINOCEPTIVE SITES

by David Wells Snyder

Two pharmacologically distinct postganglionic cholinoceptive sites have been identified in the mammalian sympathetic ganglion. The two sites will be referred to as the C_6-sensitive site and the atropine-sensitive site since the discharges evoked at these receptors are blocked by hexamethonium (C_6) and atropine respectively. This study deals with the mechanisms involved with the disparity between the ability of single injections and infusions of acetylcholine (ACh) and tetramethylammonium (TMA) to evoke C_6-sensitive and atropine-sensitive discharges.

Neurogenic- or drug-evoked potentials were recorded from acutely or chronically denervated superior cervical ganglion of cats. Drugs were administered either intravenously or directly into the blood supply of the ganglion.

A single injection of TMA evoked a postganglionic discharge that was blocked only by hexamethonium. In contrast, the discharge evoked during the infusion of TMA was blocked by either C_6 or atropine. Transmission was not altered by the infusion rates of TMA employed.

The data suggest that infusion of TMA initiated an interaction between the C_6-sensitive site and the atropine-sensitive site. The infusion of TMA evoked a well maintained C_6-sensitive depolarization, the amplitude of which was

25 to 50% of control spike height. Hexamethonium simultaneously repolarized the ganglion and blocked the postganglionic discharge evoked by infused TMA. Atropine blocked the discharge but failed to repolarize the ganglion. In a few animals a small atropine-sensitive discharge was observed following the administration of C_6.

Thus it is proposed that the spread of depolarization from the C_6-sensitive site to the atropine-sensitive site greatly facilitated the weak muscarinic stimulating properties of TMA. The postganglionic discharge evoked during the infusion of TMA appeared to emanate mainly from the atropine-sensitive site. It is proposed that atropine blocked the discharge at the site of initiation of the asynchronous action potentials whereas C_6 blocked the discharge by eliminating the spreading facilitatory depolarization.

Nicotine, administered either by single injections or constant infusion evoked postganglionic activity that was blocked by C_6 and unaffected by atropine. Depolarization comparable to that evoked by infused TMA was observed with nicotine infusion. The failure to demonstrate a muscarinic stimulating action of nicotine indicates that continuous depolarization of the C_6-sensitive site cannot alone evoke action potentials from the atropine-sensitive site.

Single injections of ACh evoked only C_6-sensitive firing in an unconditioned control ganglion. A constant infusion of the compound usually elicited a discharge which

was blocked by atropine and unaffected by C_6. The threshold of activation of the two cholinoceptive sites appeared to be reversed by infusion of ACh in an unconditioned ganglion. The possible mechanisms underlying the changes in sensitivity of the two cholinoceptive sites to TMA and ACh during infusion of these compounds were discussed.

FACILITATORY INTERACTION BETWEEN PHARMACOLOGICALLY DISTINCTIVE GANGLIONIC CHOLINOCEPTIVE SITES

By

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A THESIS

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

Department of Pharmacology

1969

356317
6-24-87

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. G. L. Gebber for his interest, valuable assistance and the constructive criticism offered throughout this investigation.

He wishes to thank Drs. T. M. Brody, R. A. Pax and J. B. Hook for their helpful and constructive assistance in the preparation of this thesis.

He is very grateful for the competent technical assistance of Mrs. Phyllis A. Whetter.

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INTRODUCTION

A. Acetylcholine and Ganglionic Transmission

The first significant evidence that acetylcholine might be a chemical mediator for transmission was demonstrated by Otto Loewi (1921; cited by Brown, 1937) in the frog heart muscle. Loewi demonstrated that the effluent collected from a perfused heart during vagal stimulation mimicked the response evoked by vagal stimulation when injected into a second frog heart. The substance released during vagal stimulation was pharmacologically identified as acetylcholine (ACh). Studies in other areas of the nervous system were undertaken in the 1930's and it was concluded that transmission in the mammalian sympathetic ganglion was chemically mediated. Kibjakow (1933; cited by Brown, 1937) developed the technique for perfusing the superior cervical ganglion of the cat. During preganglionic stimulation the venous effluent from the superior cervical ganglion could be collected and assayed for acetylcholine-like activity by the bioassay technique developed by Chang and Gaddum (1933). Using these techniques, an acetylcholine-like substance was shown to be released from the ganglion during stimulation of the preganglionic nerve trunk (Feldberg and Gaddum, 1934; Feldberg and Vartiainen, 1934). The quantity of ACh liberated during preganglionic stimulation was shown to be sufficient to elicit a postganglionic discharge (Feldberg and Gaddum, 1934). Reinjection of the

effluent collected during preganglionic stimulation evoked a similar response (contraction of the nictitating membrane) in the resting ganglion.

The proposal that the site of liberation of ACh during preganglionic stimulation was the terminals of the preganglionic sympathetic trunk and not the postganglionic elements was supported by the following: 1) Liberation of ACh during preganglionic stimulation continued unabated when transmission was blocked by physostigmine, nicotine, curare, or excess potassium (Feldberg and Vartiainen, 1934; Brown and Feldberg, 1936). 2) Acetylcholine was not detected in the venous effluent of the perfused ganglion during vagal stimulation or during ganglionic activation induced by stimulation of the postganglionic nerve (Feldberg and Vartiainen, 1934). Nicotine- or potassium-induced ganglionic activation failed to release ACh from the ganglion (Brown and Feldberg, 1936). 3) Chronic degeneration of the preganglionic trunk significantly reduced the ganglionic content of ACh (Brown and Feldberg, 1936). From these experiments it can be concluded that ACh was contained within and was released from the preganglionic nerve.

Evidence is available for the presence of both enzyme systems involved in the synthesis and metabolism of ACh in the sympathetic ganglion. Physostigmine, an anticholinesterase agent, was shown to potentiate the effects of repetitive submaximal stimulation of the preganglionic nerve in the superior cervical ganglion (Feldberg and Gaddum,

1934). This experiment demonstrated the presence of a system capable of inactivating ACh. Brown and Feldberg (1937) reported that the superior cervical ganglion has the capability of synthesizing ACh. This was later confirmed by Kahlson and MacIntosh (1939) and Birk and MacIntosh (1961). Thus, these data provided some reasonable evidence to establish ACh as the mediator of neurohumoral transmission in the sympathetic ganglion.

B. Ganglionic Cholinoceptive Sites

Acetylcholine released during preganglionic stimulation was thought to diffuse across the synaptic cleft to excite the postsynaptic cholinoceptive site. The classical cholinoceptive site involved in transmission is blocked by hexamethonium (C_6), a competitive ganglionic blocking agent (Paton and Perry, 1953). However, cholinoceptive sites other than the classical C_6-sensitive site have been proposed in the superior cervical ganglion. Koppanyi (1932) demonstrated that the sympathetic ganglion possesses more than one type of cholinergic receptor site. Koppanyi reported that mydriasis was observed following application of pilocarpine to the surface of the cat's superior cervical ganglion. Pretreatment of the ganglion with atropine abolished the response. Twenty years elapsed before significant evidence for the existence of multiple cholinoceptive sites in the ganglion was reported.

R. M. Eccles (1952a, b) investigated the response elicited by preganglionic stimulation in the curare

pretreated superior cervical ganglion of the rabbit. Following blockade of spike generation, preganglionic stimulation evoked a triphasic response. A depolarizing synaptic potential (N wave) was followed by a hyperpolarizing potential (P wave). In turn, the P wave was followed by a second and more prolonged depolarizing potential, late negative, LN wave, (Eccles, 1952b). Eccles reasoned that these were synaptic potentials and not afterpotentials since spike initiation was blocked by curare.

Eccles and Libet (1961) used the same preparation to determine the nature of these three postsynaptic potentials. They reported that after the administration of botulinum toxin the potentials elicited by preganglionic stimulation were progressively blocked. They concluded that the N, P and LN slow waves were mediated by ACh released from the preganglionic terminals. Atropine was administered to determine if more than one type of cholinergic receptor was activated by endogenously released ACh. Doses of atropine which failed to reduce the N potential, abolished the P and LN waves. Following the administration of N,N-dibenzyl-β-chloroethylamine (dibenamine), Eccles and Libet demonstrated that the slow P wave was more sensitive to blockade, suggesting the ganglionic release of catecholamines. They also reported that high concentration of curare blocked specifically the N potential, leaving a large P and LN slow waves. In a ganglia pretreated with a

high concentration of curare, anticholinesterase was shown to suppress the LN wave while the P wave increased.

To explain these results, Eccles and Libet postulated that the ganglion contained multiple cholinoceptive sites. The scheme they proposed is as follows: three cholinoceptive sites are contained in the mammalian sympathetic ganglion. 1) Acetylcholine initiated a depolarizing synaptic potential, N wave at the N receptor site. The N potential was blocked by curare and little affected by atropine. 2) Acetylcholine evoked a slow long lasting depolarization, LN wave, at the LN receptor site. The LN potential was specifically blocked by atropine. 3) The slow hyperpolarizing P wave was initiated by the actions of catecholamines at a P receptor site. Eccles and Libet suggested that ACh released from the preganglionic nerve activated an atropine-sensitive site on ganglionic chromaffin cells that affected the release of catecholamines. The catecholamines released from the chromaffin cells were believed to diffuse to the P receptor sites on the ganglion to elicit the slow hyperpolarizing potential. The actions of the catecholamines on the P receptor sites were prevented by dibenamine.

The experiments of Volle and his colleagues have demonstrated that spike initiation can occur as the result of activation of the atropine-sensitive cholinoceptive ganglionic sites. Volle (1962) described the actions of ganglionic blocking agents on the postganglionic discharge

elicited after the administration of an anticholinesterase, diisopropyl phosphorofluoridate, DFP, (Volle and Koelle, 1961). Atropine blocked this asynchronous discharge. Classical ganglionic blocking agents, hexamethonium and d-tubocurare, did not alter the discharge. Physostigmine and neostigmine were shown to produce the same characteristic asynchronous firing (Takeshige and Volle, 1962; 1963a). Volle reasoned that an atropine-sensitive site in the ganglion had been unmasked by an action of the anticholinesterase. Volle postulated that the discharge resulted from the accumulation of endogenously released ACh which activated this previously masked atropine-sensitive site.

In addition, Volle and his associates demonstrated that the intraarterial administration of ACh directly to the superior cervical ganglion of the cat activated two excitatory cholinoceptive sites. Takeshige and Volle (1962) demonstrated that following the conditioning procedures of either high frequency preganglionic stimulation (30 cps for 30 sec.) or physostigmine pretreatment, a bimodal response to exogenously administered ACh was recorded postganglionically. The two component discharge consisted of an "early" response which was blocked by C_6 or curare and a "late" response which was unaffected by C_6 and abolished by small doses of atropine. Atropine had no effect on the postganglionic

action potential elicited by preganglionic stimulation and did not alter the "early" response to ACh.

The bimodal response could be elicited in an unconditioned ganglion with a high dose of ACh (Takeshige and Volle, 1962). These experiments demonstrated that more than one type of cholinergic postsynaptic receptor site was activated by exogenously administered as well as endogenously released ACh.

Volle and his associates demonstrated that exogenously administered ACh evoked a characteristic complex change in the ganglionic demarcation potential which was similar to that elicited by preganglionic stimulation in a ganglion pretreated with curare (Takeshige et al., 1963; Takeshige and Volle, 1964; Eccles and Libet, 1961). A triphasic slow potential was elicited following the administration of ACh. Initially a depolarization (D potential) which coincided with a postganglionic discharge was observed. This was followed by a slow hyperpolarization (H potential) which corresponded to a depression of transmission. A late slow depolarizing wave (LD potential) followed the H potential. The D potential and the postganglionic discharge were blocked by C_6. The H potential and the LD potential were blocked by atropine.

The response was dose dependent since a smaller dose of ACh evoked only the H and LD potentials while a large dose elicited a prolonged D potential. The prolonged depolarization coincided with blockade of transmission but

was not related to the blockade (Takeshige and Volle, 1964).

In contrast to the triphasic potential evoked by ACh, Takeshige et al., (1963) reported a biphasic change in the demarcation potential following the intraarterial injection of acetyl-β-methylcholine (methacholine, MCh) directly to the ganglion. A hyperpolarizing wave (H potential) was followed by a late occurring depolarizing wave (LD potential). The hyperpolarization of the ganglion cells was associated with a depression of transmission. No change or perhaps an increase in transmission was associated with the LD potential. A postganglionic discharge occurred during the LD potential after the administration of methacholine. The slow potentials, postganglionic discharge and effects on transmission were abolished following the administration of a small dose of atropine.

Nicotine on the other hand, has been shown to evoke a brief depolarizing potential and a postganglionic discharge when administered directly to the ganglion (Lundberg and Thesleff, 1953; Paton and Perry, 1953). The depolarizing wave paralleled exactly the initial depolarization evoked by ACh. Hexamethonium abolished the depolarization and the postganglionic discharge evoked by nicotine. Atropine did not alter the response.

In view of these results Volle (1966) has classified cholinomimetic agents that stimulate sympathetic ganglia on the basis of their susceptibility to blockade either by

C_6 or atropine. Drugs related to nicotine (e.g., tetramethylammonium) evoked ganglionic depolarization and firing that was immediate in onset and blocked by C_6. Those substances related to muscarine (e.g., pilocarpine and methacholine) evoked responses that were delayed in onset and prevented by atropine. By contrast, acetylcholine was capable of activating both atropine- and C_6-sensitive cholinoceptive sites in the ganglion.

To explain the actions of these various drugs in the superior cervical ganglion Takeshige et al. (1963) and Takeshige and Volle (1964) have presented the following model of heterogeneity of cholinoceptive ganglionic sites. Acetylcholine activated a C_6-sensitive receptor site to elicit the initial depolarization and postganglionic discharge corresponding with this depolarization. This same site was assumed to mediate transmission since the transmission process was blocked by C_6 (Paton and Perry, 1953). An atropine-sensitive receptor site activated by ACh evoked a hyperpolarization and a corresponding decrease in transmission. A second atropine-sensitive receptor site evoked the late occurring depolarization (LD potential) following the administration of ACh or MCh. This second atropine-sensitive site elicited a postganglionic discharge following the administration of an anticholinesterase agent or MCh.

This model was very similar to the one proposed by Eccles and Libet (1961). The D potential appeared to be

identical with the N wave, the depolarizing synaptic potential. Both responses were sensitive to hexamethonium. The H potential corresponded with the P wave, both hyperpolarizing and atropine-sensitive. However, Takeshige et al. (1963) demonstrated that the H potential could be elicited in a ganglion pretreated with reserpine. This demonstrated that one type of cholinergic receptor could, upon activation by ACh, elicit a hyperpolarizing slow potential. Therefore ganglionic release of catecholamines, as proposed by Eccles and Libet (1961) would not necessarily be involved in the hyperpolarization. The late-occurring depolarization (LD potential) can be equated to the LN wave of Eccles and Libet. As was the case with the LN potential, the LD potential was blocked by atropine.

Libet and Tosaka (1966, 1969) have demonstrated that three different kinds of cholinoceptive sites are located on one sympathetic ganglion cell. They reported a triphasic slow potential in the rabbit superior cervical ganglion during preganglionic stimulation, while recording intracellularly from a single neuron. An initial depolarization was followed by a slow hyperpolarizing potential and a slow depolarizing potential. The initial depolarization was blocked by C_6. The slow potentials following the initial depolarization were selectively blocked by atropine and unaffected or increased slightly by C_6.

In summary, three different types of postsynaptic cholinergic receptor sites have been demonstrated to exist

in a single mammalian sympathetic ganglion cell (Libet and Tosaka, 1966, 1969). The one evoking the hyperpolarizing potential, which is abolished by atropine, can be considered as an inhibitory site, since transmission is depressed during this hyperpolarization. The other two, therefore, are excitatory in nature since they evoke a depolarizing potential when activated by ACh. The excitatory receptor site whose response is blocked by hexamethonium will be referred to as the C_6-sensitive site. Correspondingly the other excitatory site, blocked by atropine will be designated the atropine-sensitive site.

METHODS

All experiments were performed on the superior cervical ganglion of cats of both sexes weighing 2-4 kg. The cats were anesthetized by the intraperitoneal administration of a mixture of sodium diallybarbiturate (70 mg/kg), urethane (280 mg/kg) and monoethylurea (280 mg/kg).

The cat was placed in the supine position and was secured to a dissecting table. To keep the head and neck stationary, a mouth clamp was attached to the lower jaw and secured to the metal frame of the cat board. A midline incision was made from the symphysis of the lower jaw to the sternal notch. To insure the patency of the respiratory pathway, the trachea was cannulated at the level of the clavicle.

The superior cervical ganglion and associated structures were exposed by inverting the upper portions of the trachea, larynx and esophagus into the animal's mouth. The left superior cervical ganglion and the external carotid postganglionic nerve were prepared for recording following removal of the surrounding connective tissue. Care was taken so that the small blood vessels supplying the ganglion were not disturbed. A silk ligature, soaked in saline (0.9% NaCl) was tied to the postganglionic nerve near its junction with the external carotid artery. The nerve was then sectioned between the silk tie and the artery.

The cervical sympathetic trunk was dissected free from the carotid sheath approximately 3 centimeters from the ganglion. The cervical trunk was tied with a silk ligature and cut at the level of the clavicle. A deep cervical well was formed by tying skin flaps to the metal framework and the exposed area was covered with mineral oil. Loops were formed in the silk ligatures and suspended in the oil on glass hooks which were fastened to the metal framework.

Bipolar electrodes of 26 guage platinum wire were used for stimulating the decentralized ganglion. They were positioned on the isolated preganglionic trunk approximately 2 centimeters from the ganglion (fig. 1). Electrical stimulation was provided by a Grass model S-8 square wave generator led through a Grass model SIU-4678 stimulus isolation unit to the bipolar electrodes. Supramaximal stimuli, 15 volts, of constant duration (0.1 msec.) were employed with a frequency noted for the particular experiment.

Drug- or neurogenically-induced changes in the demarcation potential of the decentralized ganglion were recorded with silver-silver chloride bipolar electrodes. These electrodes were prepared from bright silver wire by electrolytic deposition of chloride from an acidified 0.1N KCl solution. The electrodes were replated after visible damage to the silver chloride precipitate had occurred. One pole of the electrode was placed in direct

Figure 1. Schematic drawing of the electrophysiological recording set up of the superior cervical ganglion.

Asynchronous activity was recorded with bipolar electrodes placed on the crushed end of the postganglionic nerve and an intact portion of the same nerve (lower R). Demarcation potential was recorded with bipolar electrodes placed on the surface of the ganglion and the crushed end of the postganglionic nerve (upper R). SCG - superior cervical ganglion. Pre - preganglionic nerve with bipolar stimulating electrodes. Post - postganglionic nerve. CCA - common carotid artery.

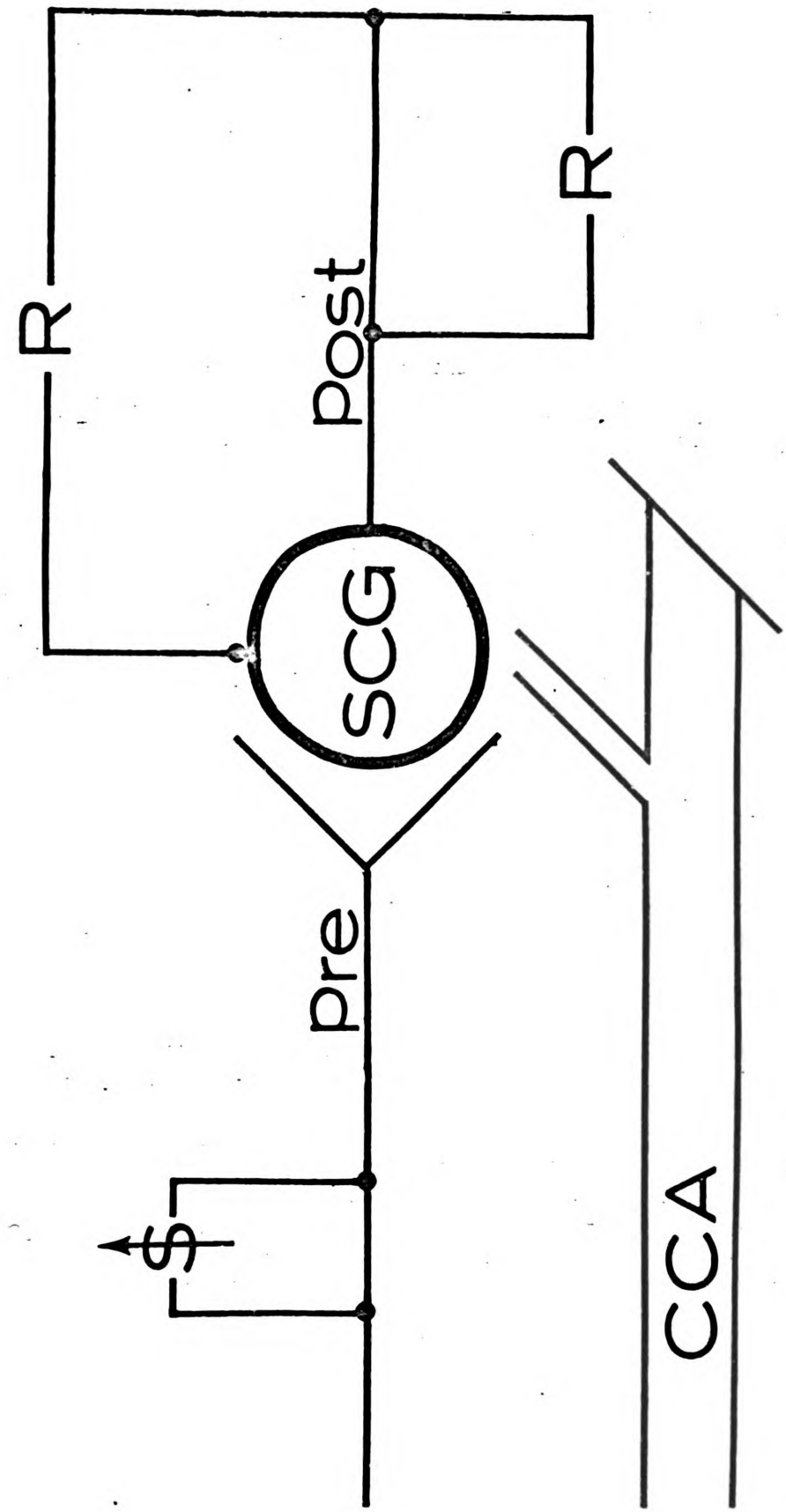

Figure 1. Schematic drawing of the electrophysiological recording set up of the superior cervical ganglion.

contact with the surface of the ganglion and the other pole was placed on the crushed end of the external carotid nerve (fig. 1). The recorded potentials were led to a cathode follower circuit that in turn led to a resistance-coupled preamplifier (Grass Model P-612, DC preamplifier). The preamplifier's low and high amplitude frequency controls were set at DC and 2000 KC respectively. The evoked potentials were displayed on a dual beam oscilloscope (Tektronix Type 502).

The demarcation potential or surface potential was a crude extracellular recording of the resting membrane potential. Drug- or neurogenically-induced changes in the potential of the ganglion cells were monitored using the crushed end of the postganglionic nerve as the reference point. An upward deflection of the demarcation potential tracing indicated ganglionic negativity (i.e., depolarization) in all records. Similarly a sudden downward shift in the tracing denoted repolarization.

To record drug-induced postganglionic action potentials platinum electrodes were used. One pole of the bipolar electrode was placed on the crushed end of the external carotid nerve. The other pole was positioned on an intact portion of the external carotid nerve. The asynchronous burst of action potentials were amplified by a capacitance-coupled preamplifier (Grass Model P-511, AC preamplifier). The half amplitude frequency controls of the amplifier's low and high coupling filters were set at 30 cps and

1000 KC, respectively. The potentials were visualized on the dual beam oscilloscope. Changes in the demarcation potential of the ganglion and the asynchronous ganglionic discharge were monitored simultaneously in some experiments. Permanent records were made on moving photographic paper (Kodak Kind 1732) with a Kymograph camera (Grass Model C4L).

In a number of experiments, movement of the left nictitating membrane was monitored in conjunction with neural recordings from the left external carotid nerve. Initial tension of the membrane was set at 7 grams and was recorded with a force-displacement transducer. Neural recording from the external carotid nerve did not compromise the innervation to the nictitating membrane. In these experiments nerve action potentials and tone of the nictitating membrane were recorded with a Grass Model 7 pen-writing polygraph. The half amplitude response of the preamplifier was set at 10 and 75 cps for the neural recordings.

Chronically denervated ganglia were studied in one series of experiments. Resection of the left vagosympathetic trunk, approximately 2 centimeters from the ganglion was performed under near sterile conditions. These cats were anesthetized with pentobarbital sodium (30 mg/kg, i.p.) prior to surgery. One centimeter of the vagosympathetic trunk was removed. The animals were

allowed to recover and the experiments were run 7-54 days following resection of the preganglionic nerve.

All major branches of the common carotid artery except those directly supplying the ganglion were tied. Single doses of drugs were administered directly to the ganglion through a 27 guage needle inserted into the common carotid artery. The injection apparatus was clamped to the metal framework. Clotting in the needle was prevented by administering heparin (300 units/kg, i.v.). The intra-arterial injection volume for a single dose was 0.1 ml. A catheter was placed in the femoral vein to infuse various drugs. Drugs were infused by the intraarterial or intravenous route with the aid of a constant rate infusion pump. The infusion volume was 0.1 to 1.0 ml/min because the concentration of drugs to evoke a postganglionic discharge varied from animal to animal. The criteria used in determining the concentration of the ganglionic stimulants employed in each experiment was such to produce a minimal effect on transmission.

To avoid movement of the recording electrodes during the experiment, the animals were paralyzed with decamethonium bromide (0.5-0.75 mg/kg, i.v.) and placed on artificial respiration. This prevented any spontaneous muscle twitches. Additional doses of decamethonium were administered as required throughout the experiment. This neuromuscular blocking agent had little effect on the responses of the ganglion evoked by drugs or preganglionic

stimulation. Blood pressure was monitored from the femoral artery with a Statham P-23 series pressure transducer and recorded on the Grass Polygraph.

The following drugs were used: acetylcholine chloride (ACh), tetramethylammonium chloride (TMA), nicotine salicylate, hexamethonium chloride (C_6), and atropine sulfate. All drugs were dissolved in 0.9% saline. All doses are expressed in terms of the salt.

RESULTS

I. CHARACTERIZATION OF POSTGANGLIONIC ACTIVITY EVOKED BY TMA, ACh AND NICOTINE

A. TMA Infusion

Infusions of TMA (50-200 µg/kg/min. i.v,; 5-20 µg/min. i.a.) elicited a low amplitude, asynchronous postganglionic discharge. In 25 experiments, the discharge rapidly reached and was maintained at or near peak amplitude for the duration of the infusion. A typical postganglionic response induced by the infusion of TMA is illustrated in the TMA record of fig. 2. Line 1 of fig. 2 illustrates the control background of the acutely denervated, unstimulated ganglion. The low amplitude asynchronous ganglionic activity evoked by infused TMA is demonstrated by the increased width of the record in line 2 of fig. 2. The functional significance of the low amplitude discharge was demonstrated by monitoring contractions of the nictitating membrane. In four experiments, the amplitude of the contraction of the nictitating membrane during the infusion of TMA equalled that evoked by supramaximal stimulation of the preganglionic nerve at frequencies from 1-3 impulses per second. A comparison of the drug- and neurogenically-induced contraction of the nictitating membrane is illustrated in fig. 3. Crushing the ganglion eliminated 80-95% of the response of the

Figure 2. Comparison of the action of C_6 and atropine on postganglionic discharge evoked by infusions of TMA and nicotine.

TMA record: I, control, background activity recorded from unstimulated postganglionic nerve; II, effect of C_6 (1 mg, i.a.) on postganglionic discharge evoked by i.v. infusion of TMA (130 µg/kg/min); C_6 was administered 3 min after initiation of the discharge; III, effect of atropine (2 µg, i.a.) on postganglionic discharge which was re-initiated 60 min after administration of C_6. Nicotine record: I, control background; II, effect of C_6 (1 mg, i.a.) on postganglionic discharge evoked by i.v. infusion of nicotine (70 µg/kg/min); III, effect of atropine (2 µg, i.a.) on discharge which was re-initiated 50 min after administration of C_6. TMA and nicotine records are from different experiments. Vertical calibration is 10 µV. Horizontal calibration is 4 sec. Dot under each record indicates time of injection of C_6 or atropine.

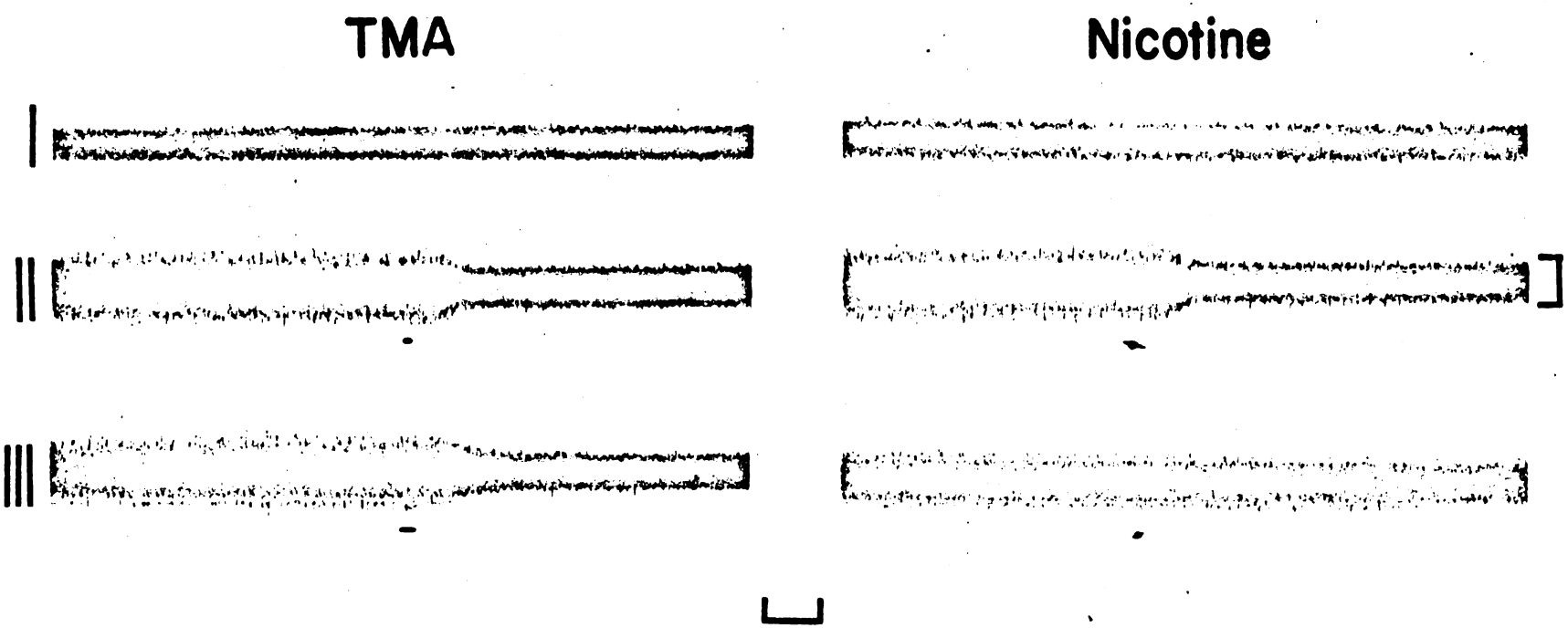

Figure 2. Comparison of the action of C_6 and atropine on postganglionic discharge evoked by infusions of TMA and nicotine.

Figure 3. Comparison of the ganglionic response evoked by infusion of TMA and preganglionic stimulation.

Upper record, contraction of the nictitating membrane (top) and asynchronous postganglionic discharge (bottom) evoked by the i.v. infusion of TMA (67 µg/kg/min). Lower record, contraction of the nictitating membrane (top) and compound ganglionic action potentials (bottom) evoked by supramaximal stimulation of the preganglionic nerve (2 cps). Nerve action potentials were amplified with a Grass 7P3A preamplifier and displayed on a pen-writing polygraph with half-amplitude response at 10 and 75 cps. Vertical calibrations referring to neural activity are 25 µV (upper record) and 1 mV (lower record). Vertical calibration referring to contraction of nictitating membrane is 10 grams. Time base is 1 sec per division. Downward deflection of time base in upper record indicates start of TMA infusion.

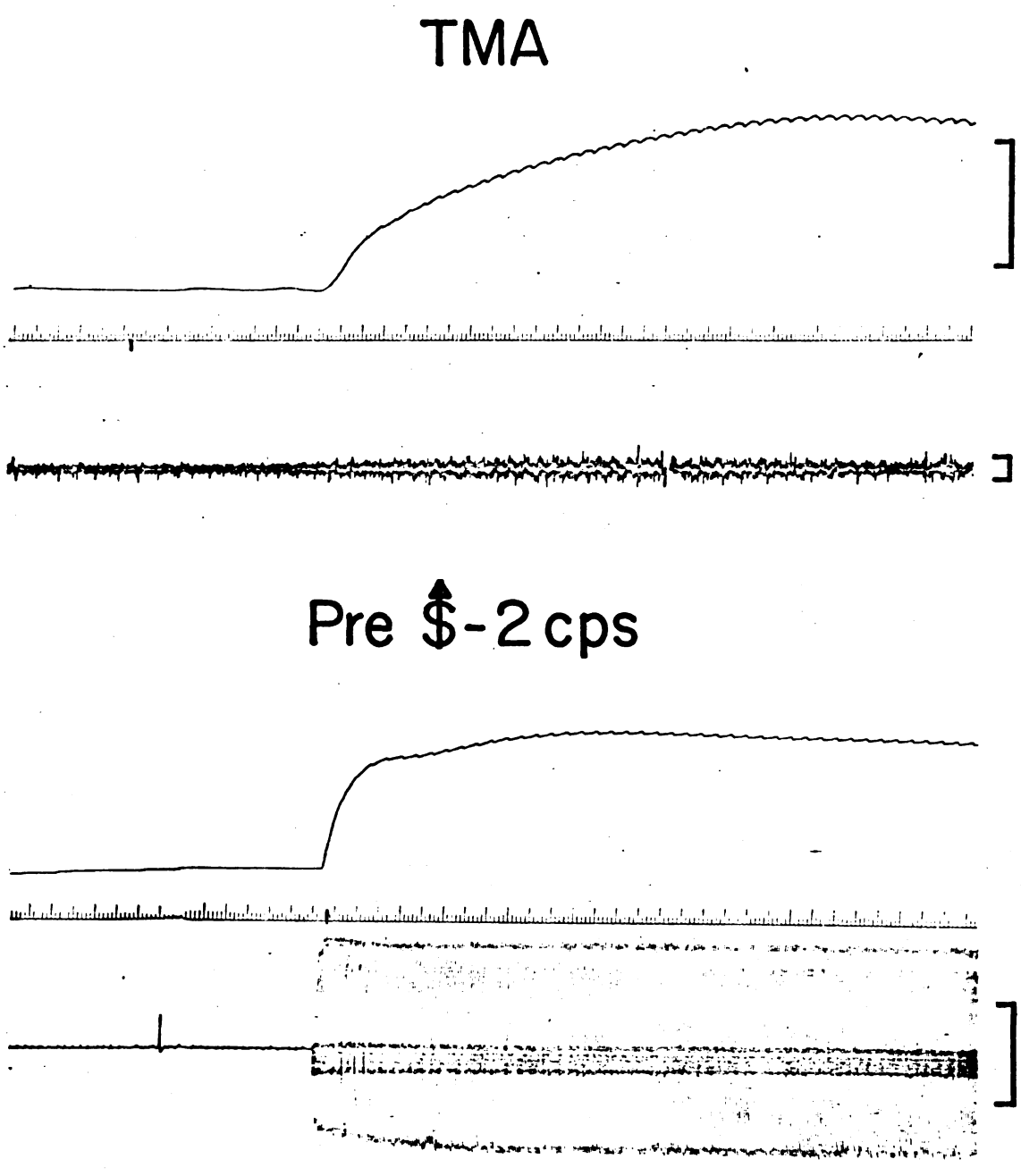

Figure 3. Comparison of the ganglionic response evoked by infusion of TMA and preganglionic stimulation.

nictitating membrane. Thus, the contraction evoked by TMA was primarily of ganglionic origin.

The characteristics of the postganglionic discharge could be studied during the period of time (10-15 min.) before TMA began to block ganglionic transmission. As illustrated in fig. 4, the amplitude of the action potential was only slightly depressed at the indicated times during the infusion of TMA. This is one of four experiments in which transmission was monitored during the infusion of TMA.

B. Nicotine Infusion

Intravenous infusion of nicotine (50-100 µg/kg/min.) evoked a low amplitude, asynchronous postganglionic discharge in four experiments. The amplitude of the nicotine induced postganglionic discharge was comparable to that evoked by the TMA infusion as shown in fig. 2.

C. ACh Infusion

In nine experiments, ACh was infused into the arterial circulation of the superior cervical ganglion. Even though the intraarterial route of administration helped to minimize the systemic effects produced by ACh there was a marked fall in blood pressure during the infusion. To limit this pronounced depressor effect, ACh was never infused for more than 5-6 minutes and the dose required to produce a postganglionic discharge was kept to a minimum (40-80 µg/min.). Blood

Figure 4. Comparison of the action of C_6 and atropine on changes in transmission and the asynchronous postganglionic discharge evoked by the i.v. infusion of TMA (100 µg/kg/min).

I, control; postganglionic background (left) and ganglionic action potential evoked by supramaximal preganglionic stimulus 0.5 cps (right); II, postganglionic activity recorded 4 min after the initiation of the TMA infusion (left), transmission during the TMA infusion (right); III, effect of C_6 (1 mg, i.a.) administered 3 min after initiation of the postganglionic discharge; IV, effect of atropine (2 µg, i.a.) on the postganglionic discharge which was reinitiated 60 min after administration of C_6. Vertical calibration is 10 µV left and 1 mV right. Horizontal calibration is 4 sec. Dots under records indicate administration of C_6 or atropine.

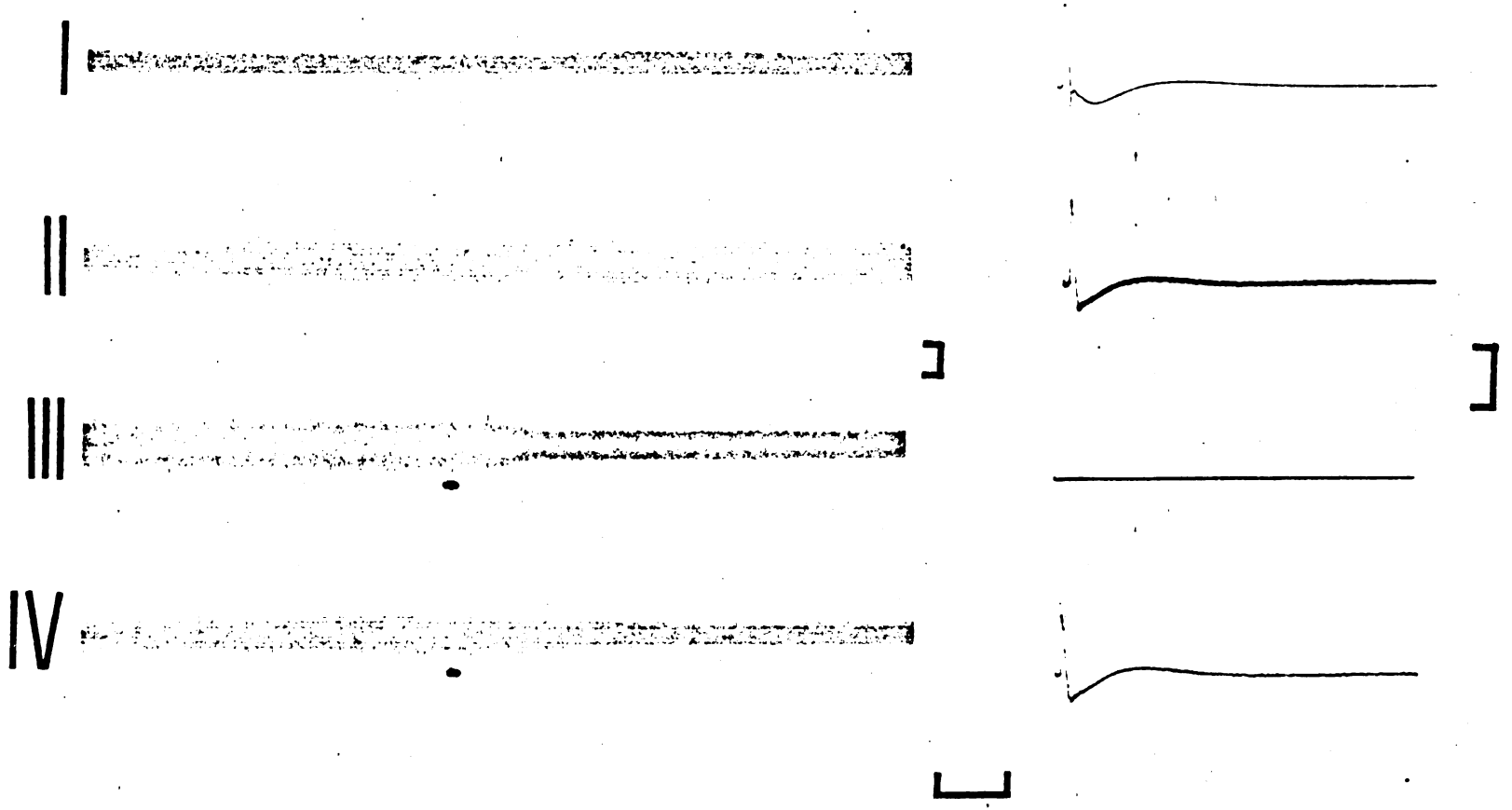

Figure 4. Comparison of the action of C_6 and atropine on changes in transmission and the asynchronous postganglionic discharge evoked by the i.v. infusion of TMA (100 µg/kg/min).

pressure quickly returned to control level upon cessation of the infusion.

The postganglionic discharge evoked during the intraarterial infusion of ACh followed one of two characteristic patterns. In six of nine experiments the asynchronous discharge was continuous and of low amplitude. The amplitude of the discharge was similar to the postganglionic discharge evoked by the infusion of TMA or nicotine. A typical ACh induced discharge of this type is illustrated in fig. 5A.

In the remaining three experiments, the postganglionic discharge evoked during the infusion of ACh followed a second pattern. The ganglionic activity appeared in characteristic bursts (fig. 5B). The bursts were of higher amplitude and shorter duration when compared with the continuous low amplitude discharge discussed previously.

D. Single Injections of TMA and Nicotine

In contrast to the low amplitude asynchronous discharge evoked by an infusion of TMA or nicotine, single intraarterial injections of TMA (1-10 µg, i.a.) and nicotine (1-10 µg, i.a.) evoked a brief discharge of relatively high amplitude in eight experiments. The discharge was rapid in onset and gradually dissipated to background level within 20 seconds as illustrated in fig. 6. In two experiments the postganglionic discharge evoked by single injections of TMA

Figure 5. Postganglionic discharge evoked by the infusion of ACh.

A: I, control background. II, administration of C_6 (1 mg, i.a.) 2 min after initiation of postganglionic discharge by ACh (60 µg/min, i.a.). III, effect of atropine (2 µg, i.a.) administered 2 min after C_6. B: I, control background. II, effect of C_6 (1 mg, i.a.) on discharge evoked by ACh (80 µg/min, i.a.). III, effect of atropine (4 µg, i.a.) on discharge. Atropine was administered 45 sec after C_6. Vertical calibration is 10 µV. Horizontal calibration is 4 sec. Dots under records indicate administration of C_6 or atropine.

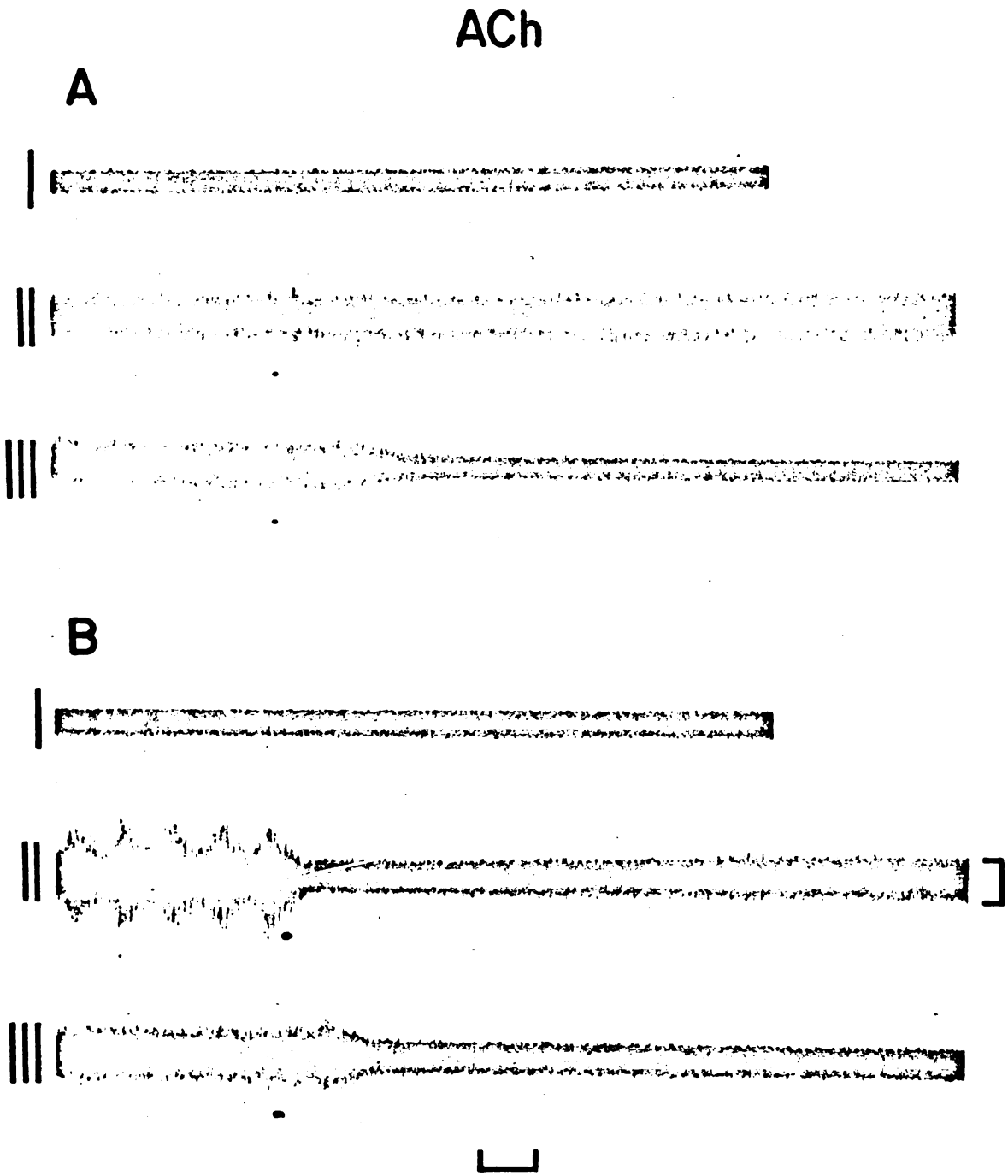

Figure 5. Postganglionic discharge evoked by the infusion of ACh.

Figure 6. Comparison of the action of atropine (4 µg, i.a.) and C_6 (1 mg, i.a.) on the postganglionic discharge evoked by nicotine (5 µg, i.a.) and TMA (5 µg, i.a.).

I, control discharges elicited by single doses of nicotine and TMA. II, discharges evoked 5 to 10 min after atropine. III, blockade by C_6 of the discharges evoked by nicotine and TMA. Vertical calibration is 10 µV. Horizontal calibration is 4 sec.

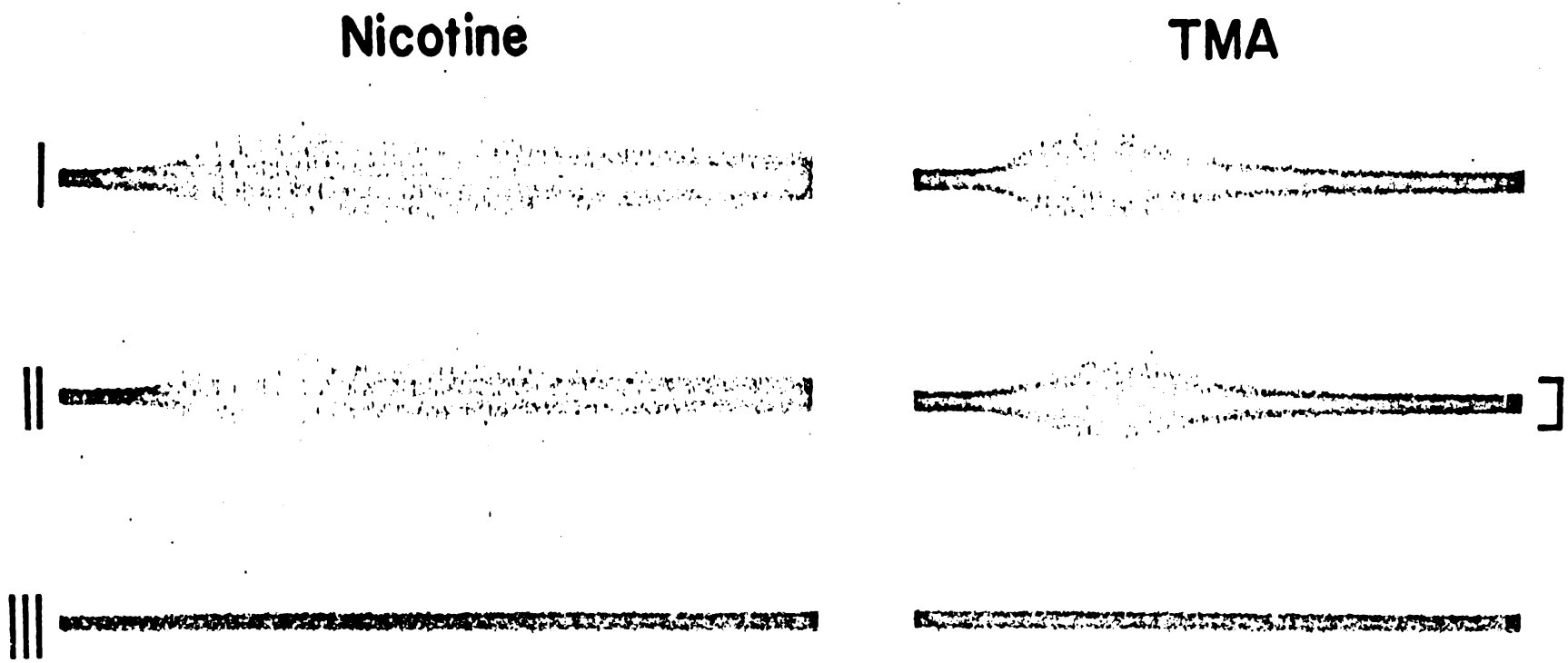

Figure 6. Comparison of the action of atropine (4 µg, i.a.) and C_6 (1 mg, i.a.) on the postganglionic discharge evoked by nicotine (5 µg, i.a.) and TMA (5 µg, i.a.).

and nicotine were enhanced following repetitive preganglionic stimulation. This observation has been previously reported by Takeshige et al. (1963); Trendelenburg and Jones (1965) and Gebber and Volle (1966).

II. PHARMACOLOGICAL BLOCKADE OF POSTGANGLIONIC ACTIVITY INDUCED BY TMA, ACh AND NICOTINE

A. TMA Infusion

The postganglionic discharge evoked by the infusion of TMA (50-200 µg/kg/min.; 5-20 µg/min., i.a.) in an acutely decentralized ganglion was markedly reduced or abolished by either C_6 or atropine. As illustrated in line 2 of the TMA record of fig. 2, C_6 (0.5-2 mg, i.a.) abolished the discharge evoked by the infusion of TMA. This was observed in each of eight experiments. These doses of C_6 completely blocked transmission. A small but perceptible postganglionic discharge reappeared 2-3 minutes after the administration of C_6. Additional doses of C_6 (1 mg) failed to alter the residual discharge. Atropine (1-4 µg, i.a.) abolished the C_6-resistant discharge.

In two additional experiments, C_6 was administered intraarterially to the ganglion, in amounts that failed to completely block ganglionic transmission. Doses of C_6 (1-5 µg, i.a.) which did not alter transmission, failed to affect the postganglionic discharge evoked

during the infusion of TMA. Similarly, doses of C_6 (20-50 µg, i.a.) which produced partial blockade of transmission produced a parallel reduction in the post-ganglionic discharge evoked by TMA infusion.

Atropine (1-4 µg, i.a.) was shown in four experiments to abolish or markedly reduce the discharge evoked by the infusion of TMA in a ganglion which had not been previously treated with C_6. These doses of atropine failed to alter transmission in the absence of TMA. However, during the infusion of TMA, atropine produced a transient (5-20 sec.) and partial block (10-20%) of transmission. This was determined from the results of two experiments. However, it should be stressed that the complete blockade of the asynchronous discharge far outlasted the transient and partial blockade of transmission produced by atropine. Following the administration of atropine, the postganglionic discharge induced by the TMA infusion could not be elicited for 2-3 hours.

These observations suggested that most or all of the postganglionic discharge initiated by the infusion of TMA could be blocked by either C_6 or atropine in any particular ganglion. Figure 2 compares the actions of atropine and C_6 on the discharge evoked by TMA in the same ganglion. The discharge was initially blocked by C_6 (1 mg, i.a.) (line 2). Following the administration of C_6 the infusion was discontinued until the effects of

C_6 had dissipated (30-60 min.) and transmission returned to control level. The amplitude of the postganglionic discharge evoked during the second TMA infusion approximated that observed during the initial period of infusion (line 3). Administration of atropine (2 µg, i.a.) abolished the discharge (line 3). The same pattern of results were observed in seven additional experiments. In three of the eight experiments performed, however, a small component of the postganglionic discharge was resistant to blockade by atropine. The small, but perceptible, atropine-resistant discharge was abolished by C_6 and unaffected by additional doses of atropine. Although increasing the infusion rate of TMA (500-1000 µg/kg/min. i.v.) quickly blocked transmission, it did not enhance this small atropine-resistant discharge.

A comparison of the actions of C_6 and atropine on transmission during the TMA infusion is illustrated in fig. 4. Atropine abolished or markedly reduced the postganglionic discharge but produced little effect on the TMA-induced ganglionic action potential evoked by preganglionic stimulation (line 4). C_6, on the other hand, abolished both the postganglionic discharge and ganglionic transmission.

B. Nicotine Infusion

C_6 abolished the postganglionic discharge evoked during the infusion of nicotine (30-100 µg/kg/min. i.v.).

In contrast to its action on the postganglionic discharge induced by infused TMA, atropine (1-4 µg, i.a.) failed to block the postganglionic discharge evoked by the infusion of nicotine. A comparison of the actions of atropine and C_6 on the discharge evoked by the infusion of nicotine is illustrated in fig. 2 which is one of four experiments performed.

C. ACh Infusion

As previously noted the intraarterial infusion of ACh (40-80 µg/min.) either evoked a continuous low amplitude postganglionic discharge (pattern one) or the postganglionic activity occurred mainly in bursts (pattern two). A comparison of the actions of C_6 and atropine on the continuous low amplitude discharge evoked during the infusion of ACh is illustrated in fig. 5A. C_6 (1 mg, i.a.) had little effect on the postganglionic discharge (line 2). Atropine (2 µg, i.a.) however abolished the discharge (line 3). The results were consistent in the six experiments in which ACh evoked a discharge similar to pattern one. After dissipation of the transmission blocking effects of C_6 (30-60 min.) an additional dose of atropine was administered (0.5 mg/kg, i.v.) to prevent the systemic depressor actions of ACh. The infusion of ACh was re-initiated at a much higher rate (200-500 µg/min, i.a.). In the presence of atropine, ACh failed to evoke a postganglionic discharge. Transmission quickly failed

at the higher infusion rates.

Figure 5B illustrates the second pattern of discharge evoked during the infusion of ACh observed in the three remaining experiments. The activity occurred mainly in bursts. C_6 (1 mg, i.a.) abolished the discharge (line 2). However, within seconds a postganglionic discharge reappeared. The discharge was abolished by atropine (4 µg, i.a.) as shown in line 3 of fig. 5B. An additional dose of C_6 failed to alter the atropine-sensitive discharge.

D. Single Injections of Nicotine and TMA

The actions of C_6 and atropine on the postganglionic activity evoked by single injections of TMA and nicotine followed that of the nicotine infusion. As illustrated in fig. 6 the postganglionic discharge evoked by single injections of TMA (1-10 µg, i.a.) and nicotine (1-10 µg, i.a.) were blocked by C_6 (0.5-1 mg, i.a.). This confirms earlier reports (Takeshige et al., 1963; Takeshige and Volle, 1964). The dose of hexamethonium used to block the drug-induced discharge also blocked ganglionic transmission. Atropine (1-4 µg, i.a.) was tested on the postganglionic discharge evoked by single doses of TMA and nicotine. The results of four experiments demonstrated that atropine failed to produce consistent changes in the postganglionic responses evoked by TMA and nicotine. These doses of atropine did not alter transmission. However, in two

experiments, larger doses of atropine (10-20 µg, i.a.) which caused a perceptible blockade of transmission produced a parallel reduction in the postganglionic discharge evoked by TMA and nicotine. Takeshige et al. (1963) previously reported a lack of specific effect of atropine on the postganglionic discharge evoked by nicotine.

III. GANGLIONIC DEPOLARIZATION EVOKED DURING THE INFUSION OF TMA

Because of the long infusion period and the inherent properties of the resistance coupled pre-amplifier to drift during this time, the changes in the ganglionic demarcation potential could not be measured directly. However, an approximate measure of ganglionic depolarization induced by an infusion of TMA could be gained from the immediate relief from depolarization produced by the administration of C_6. In a control ganglion, C_6 blocked transmission without altering the demarcation potential. Administering C_6 to a drug-induced depolarized ganglion will show a positive shift in the demarcation potential recording, demonstrating repolarization.

In five experiments repolarization of the ganglion by C_6 amounted to 25 to 50% of the amplitude of the control compound ganglionic action potential. Relief from depolarization coincided with the blockade of the

asynchronous postganglionic discharge evoked by the infusion of TMA as illustrated in fig. 7.

In contrast to the actions of C_6, atropine blocked the asynchronous postganglionic discharge induced by the TMA infusion but failed to alter the ganglionic demarcation potential. Thus, atropine failed to block TMA-induced ganglionic depolarization. One of the five experiments performed is illustrated in fig. 7.

Rapid ganglionic depolarization was demonstrated following single injections of TMA into the blood supply of the superior cervical ganglion (Gebber and Volle, 1966). However, a period of rapid depolarization was not observed at the initiation of the TMA infusion. Thus, it appeared that the C_6-sensitive depolarization gradually reached peak amplitude.

IV. GANGLIONIC RESPONSE TO INFUSION OF TMA FOLLOWING REPETITIVE PREGANGLIONIC STIMULATION

Repetitive preganglionic stimulation has been demonstrated to potentiate the stimulating actions of ACh, nicotine, TMA and methacholine in the superior cervical ganglion (Volle, 1962; Takeshige et al., 1963; Trendelenburg and Jones, 1965; Gebber and Volle, 1966). Post-tetanic potentiation at both the C_6-sensitive site and the atropine-sensitive site endures for several hours. In view of these reports and the marked effect of preganglionic tetanus on drug-induced

Figure 7. Comparison of the action of C_6 (1 mg, i.a.) and atropine (2 µg, i.a.) on changes of the ganglionic demarcation potential and postganglionic discharge evoked by the i.v. infusion of TMA (100 µg/kg/min.).

I, control; upper tracing is demarcation potential and lower tracing is postganglionic background. II, effect of C_6 administered 2 min after initiation of the postganglionic discharge of TMA. III, effect of atropine on TMA response which was reinitiated 55 min after administration of C_6. Vertical calibration refers to postganglionic activity and is 10 µV. Horizontal calibration is 4 sec. Dot in II and III is 500 µV and indicates time of administration of C_6 and atropine.

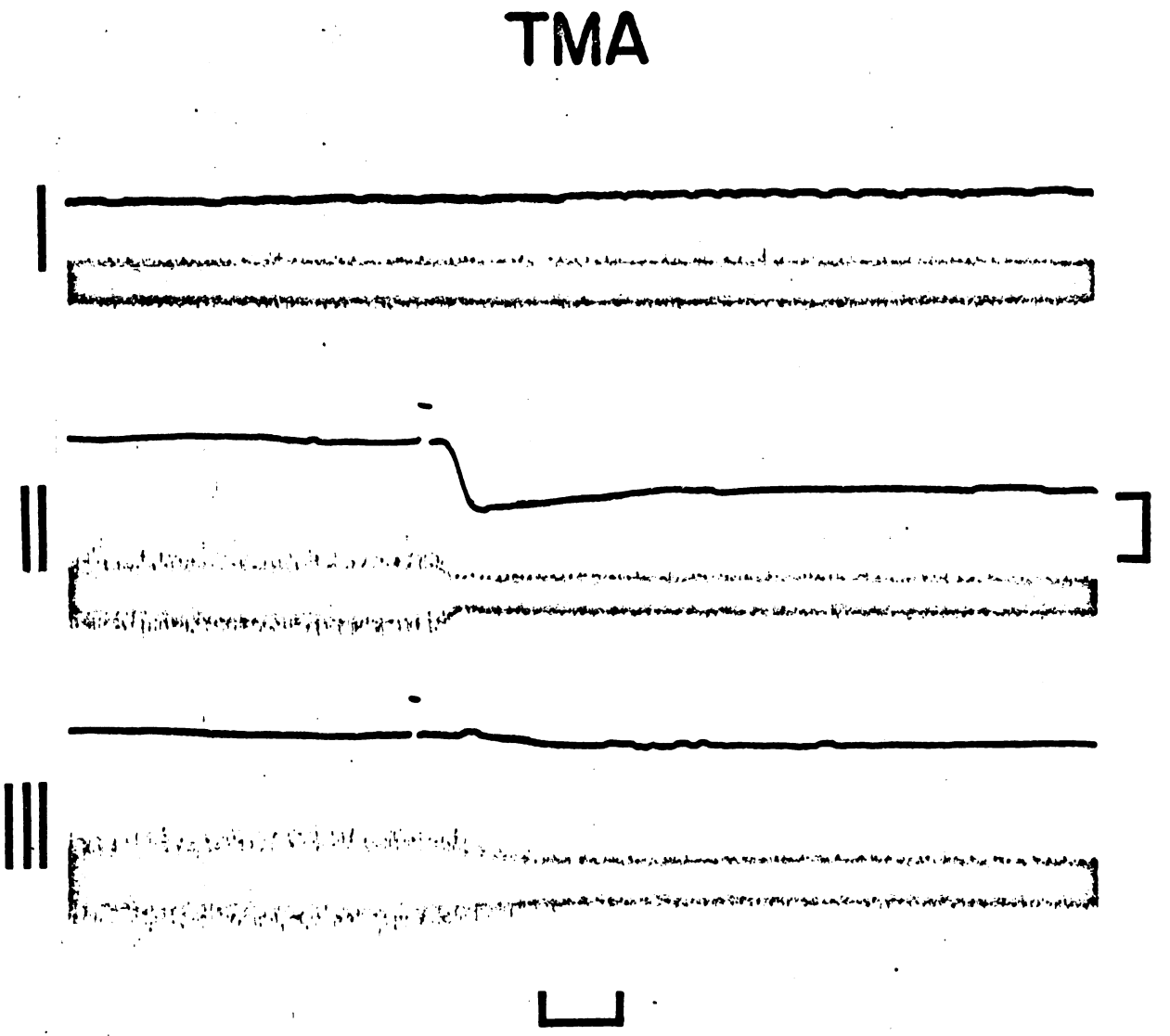

Figure 7. Comparison of the action of C_6 (1 mg,i.a.) and atropine (2 µg, i.a.) on changes of the ganglionic demarcation potential and postganglionic discharge evoked by the i.v. infusion of TMA (100 µg/kg/min.).

activation of the ganglion, it was considered important to test the effects of repetitive preganglionic stimulation on the postganglionic discharge induced by the infusion of TMA.

High frequency preganglionic stimulation (30 cps for 30 sec.) was performed during TMA infusion in eight experiments. The amplitude of the postganglionic discharge initiated by the infusion of TMA was not substantially altered by the preganglionic tetanus. However, repetitive preganglionic stimulation altered the blocking effects of both C_6 and atropine on the postganglionic discharge evoked by TMA.

In five experiments, C_6 (1 mg, i.a.) initially blocked the postganglionic discharge induced by the TMA infusion in a ganglion which had been conditioned with repetitive preganglionic stimulation (fig. 8A). In contrast to an unconditioned ganglion, however, the duration of blockade was shortened. As is illustrated in fig. 8A, the postganglionic discharge reappeared within one minute following the administration of C_6. Additional doses of C_6 failed to alter the discharge even though transmission was blocked. Administration of atropine (4 μg, i.a.) abolished the C_6-resistant discharge for the duration of the infusion.

In three experiments, the ganglionic blocking agents were administered in the reverse order in ganglia conditioned with repetitive preganglionic stimulation.

Figure 8. Effect of repetitive preganglionic stimulation on postganglionic discharge evoked by infusion of TMA.

A: I, control background; between I and II infusion of TMA (60 µg/kg/min, i.v.) was initiated and supramaximal preganglionic stimulation (30 cps for 60 sec) was performed. II, effect of C_6 (0.5 mg, i.a.) on discharge evoked by TMA in tetanized ganglion; C_6 was administered 4 min after preganglionic tetanus. III, effect of atropine (2 µg, i.a.) on discharge which returned 70 sec after administration of C_6. IV, background recorded in the continued presence of TMA infusion and 5 min after administration of atropine. B: I, control background. II, effect of atropine (4 µg, i.a.) administered 3 min after preganglionic tetanus (30 cps for 60 sec) performed during the infusion of TMA (80 µg/kg/min, i.v.). III, effect of C_6 (1 mg, i.a.) which was administered 1 min after atropine. Vertical calibration is 10 µV. Horizontal calibration is 4 sec. Calibrations refer to both experiments. Dots below records indicate time of administration of C_6 and atropine.

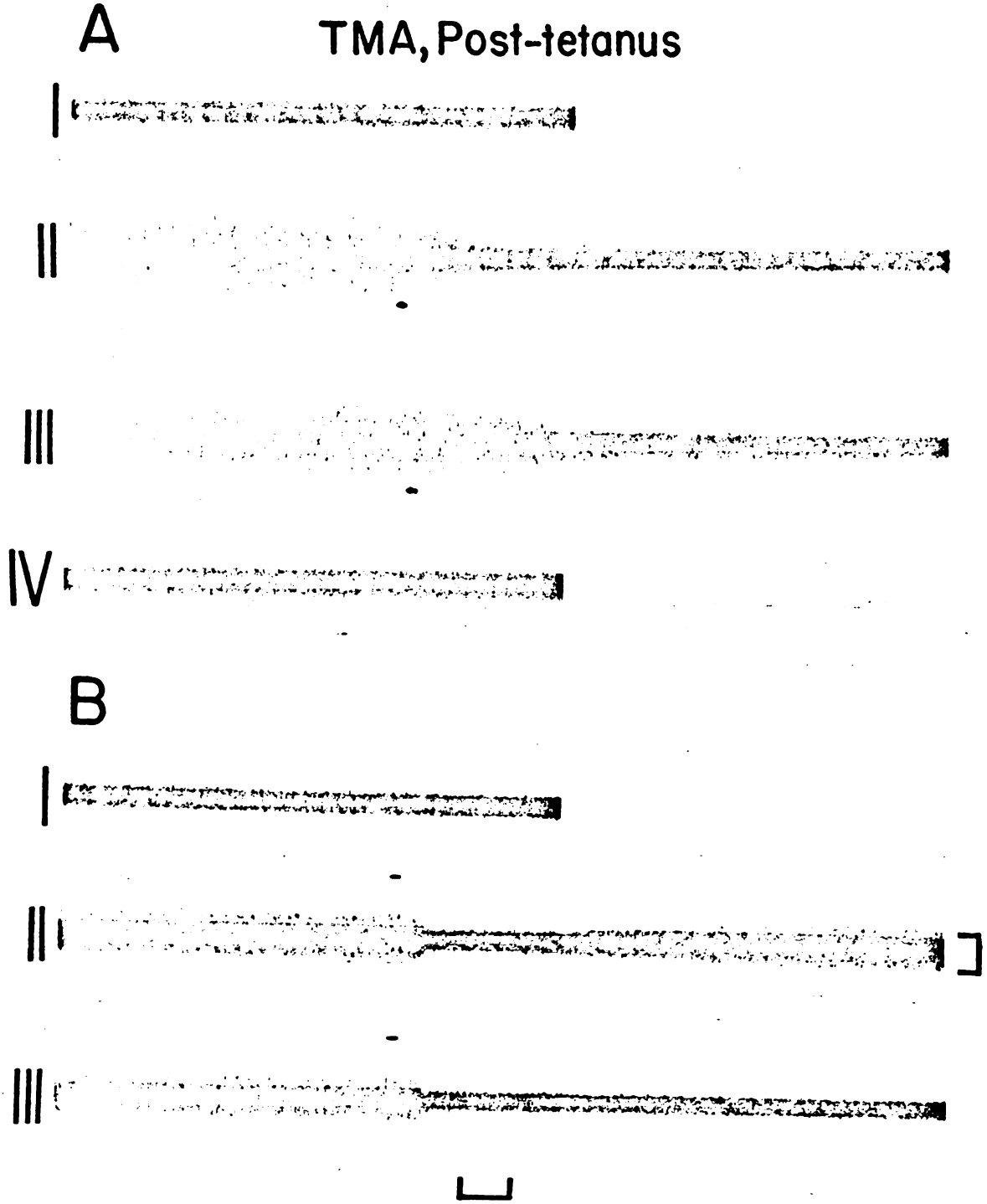

Figure 8. Effect of repetitive preganglionic stimulation on postganglionic discharge evoked by infusion of TMA.

The duration of blockade of the TMA-induced discharge by atropine (4 µg, i.a.) was also attenuated (fig. 8B). The postganglionic discharge recovered within seconds and additional doses of atropine (4 µg, i.a.) failed to alter the response. C_6 (1 mg, i.a.) abolished the discharge for the duration of the infusion. Transmission returned within 20-40 minutes following the administration of C_6 and only then could a postganglionic discharge be re-initiated by the TMA infusion.

Another series of experiments were performed to further study the effects of repetitive stimulation on the TMA discharge. In five experiments the ganglion was conditioned with repetitive preganglionic stimulation and a large dose of C_6 (5 mg/kg, i.v.) was administered which blocked transmission. The TMA infusion was then initiated. As shown in fig. 9, even though transmission was blocked, the amplitude of the postganglionic discharge evoked by the infusion of TMA fell within the range of that elicited in an unconditioned ganglion. Additional doses of C_6 (1 mg, i.a.) did not alter the postganglionic discharge. Atropine (4 µg, i.a.) abolished the discharge. Thus the atropine-sensitive discharge which was just perceptible 2-3 minutes after the administration of C_6 in a control ganglion appeared to be enhanced in ganglia conditioned with repetitive preganglionic stimulation.

Figure 9. Postganglionic discharge evoked by i.v. infusion of TMA (67 µg/kg/min) in ganglion conditioned with repetitive preganglionic stimulation (30 cps for 60 sec) and a transmission-blocking dose of C_6 (5 mg/kg, i.v.).

I, postganglionic background after preganglionic tetanus and C_6. II, effect of an additional dose of C_6 (1 mg, i.a.) on postganglionic discharge evoked by TMA. III, effect of atropine (4 µg, i.a.) administered 90 sec after i.a. dose of C_6 or 6 min after initiation of discharge by TMA. Vertical calibration is 10 µV. Horizontal calibration is 4 sec. Break in records II and III indicates time of administration of C_6 and atropine.

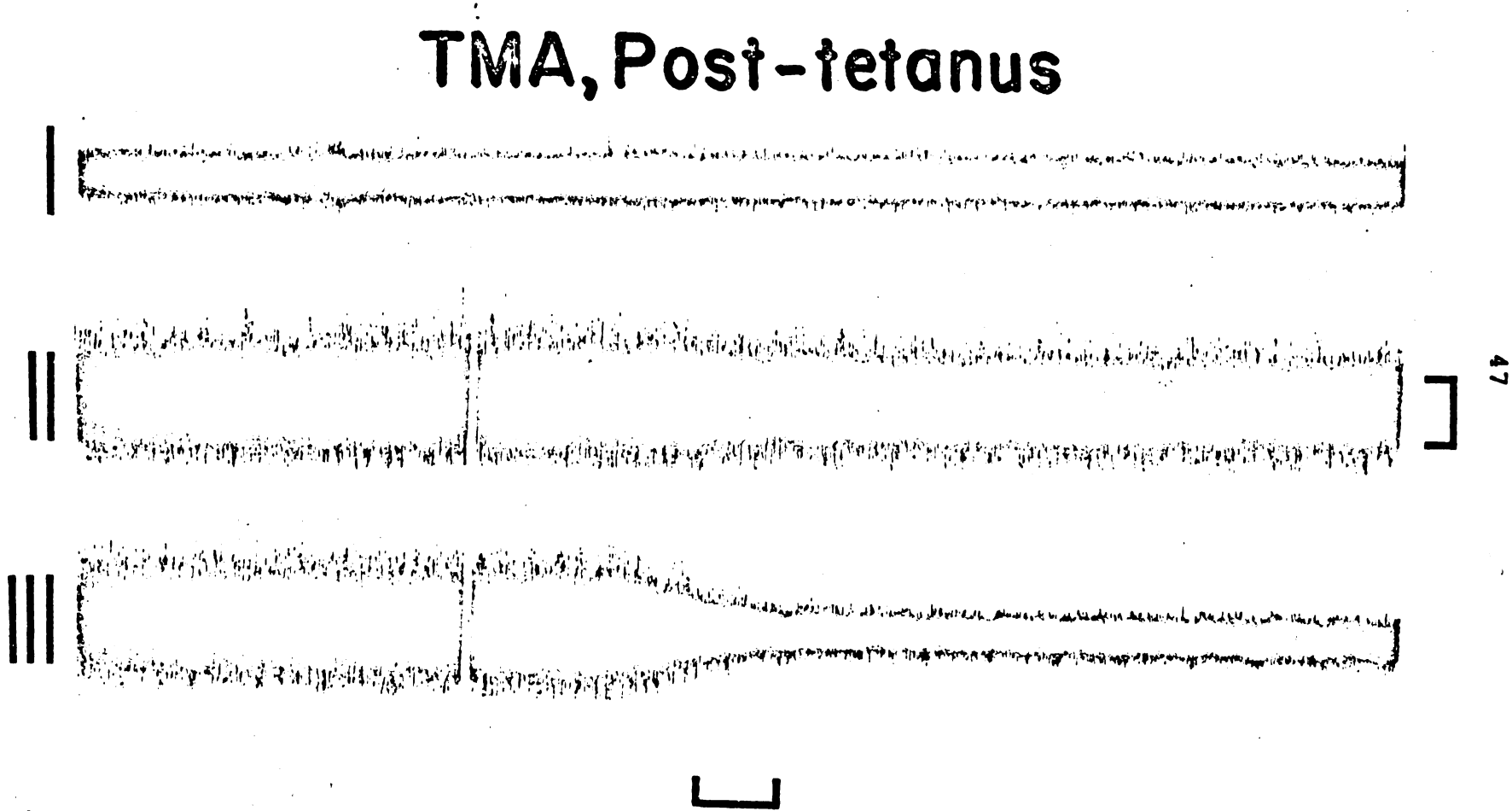

Figure 9. Postganglionic discharge evoked by i.v. infusion of TMA (67 µg/kg/min) in ganglion conditioned with repetitive preganglionic stimulation (30 cps for 60 sec) and a transmission-blocking dose of C_6 (5 mg/kg, i.v.).

Three other experiments were performed in a similar manner to those previously described. Following preganglionic tetanus, atropine (0.5 mg/kg, i.v.) was administered prior to the infusion. During the infusion of TMA a postganglionic discharge was elicited whose amplitude fell within the range observed in ganglia which had not been preconditioned with preganglionic tetanus and atropine. Additional doses of atropine (4 µg, i.a.) injected directly into the blood stream of the ganglion failed to alter the discharge. C_6 (1 mg, i.a.) abolished the postganglionic discharge. Again repetitive preganglionic stimulation appeared to enhance the small but perceptible C_6-sensitive discharge which was observed following the administration of atropine in three of eight control ganglion.

Single doses of TMA (1-10 µg, i.a.) and nicotine (1-10 µg, i.a.) were administered directly into the circulation of the ganglion conditioned with repetitive preganglionic stimulation (30 cps for 60 sec.). The preganglionic tetanus appeared to have enhanced the postganglionic discharge to single injections of TMA and nicotine. This confirms earlier reports (Takeshige et al., 1963; Trendelenburg and Jones, 1965; Gebber and Volle, 1966). In two experiments atropine (1-4 µg, i.a.) failed to reverse the enhancement of the discharges evoked by TMA and

nicotine after repetitive stimulation. Hexamethonium (1 mg, i.a.) abolished the discharge evoked by single doses of nicotine and TMA in tetanized ganglia.

V. EFFECTS OF CHRONIC DENERVATION ON GANGLIONIC RESPONSE EVOKED BY INFUSION OF TMA.

It has been reported that the atropine-sensitive postganglionic discharge evoked by single injections of ACh was not dependent upon the integrity of presynaptic terminals of the superior cervical ganglion (Takeshige and Volle, 1963b). However, the postganglionic discharge evoked by certain anticholinesterase agents was dependent on the presence of a functional presynaptic terminal (Takeshige and Volle, 1962). In addition it has been reported that the cholinergic stimulating agent, carbachol, released ACh from the preganglionic nerve terminal (McKinstry and Koelle, 1967a, b). In view of these reports, it was considered important to determine if the discharge evoked by the infusion of TMA was dependent on the integrity of the presynaptic terminal in the ganglion.

The characteristics of the postganglionic discharge evoked by TMA (50-200 µg/kg/min.) were studied 7-54 days following section of the cervical vagosympathetic trunk. In all experiments performed with chronically denervated ganglia, the amplitude of the postganglionic

discharge evoked during the infusion of TMA, was within the range observed in acutely decentralized ganglia.

TMA was infused (50-200 µg/kg/min, i.v.) in four experiments which were performed 7-11 days following nerve section. Degeneration of the preganglionic nerve appeared complete since a compound postganglionic action potential could not be elicited by stimulating the preganglionic trunk rostal to the site of resection. The discharge evoked by TMA in these experiments were very similar to those observed in the acutely decentralized ganglion. Figure 10, upper record, illustrates a typical experiment. Atropine (1-4 µg, i.a.) or C_6 (0.5-1 mg, i.a.) abolished or markedly reduced the postganglionic discharge evoked during an infusion of TMA. The duration of blockade by these ganglionic blocking agents was the same as in an unconditioned acutely decentralized ganglion.

Atropine was less effective in blocking the discharge evoked by TMA in ganglia which were denervated for 16-23 days. Typical of the four experiments performed is the one illustrated in fig. 10, lower record. As shown in line 3 of the record of a ganglion denervated for 16 days, a major component of the postganglionic discharge was resistant to blockade by atropine (1-4 µg, i.a.). C_6 (0.5-1 mg, i.a.) abolished the discharge when administered before atropine and blocked the residual discharge when administered after

Figure 10. Postganglionic discharge evoked by infusion of TMA in ganglia denervated for 7 and 16 days.

7 days: I, control background. II, effect of C_6 (1 mg, i.a.) on discharge elicited by TMA (100 µg/kg/min, i.v.); C_6 was administered 3 min after the discharge was initiated. III, effect of atropine (4 µg, i.a.) on the discharge which was reinitiated 40 min after the administration of C_6. 16 days: I, control background. II, effect of C_6 (1 mg, i.a.) on discharge evoked by TMA (100 µg/kg/min, i.v.). III, effect of atropine (4 µg, i.a.) on discharge which was reinitiated 40 min after the administration of C_6. Vertical calibration is 10 µV. Horizontal calibration is 4 sec. Dots below records indicate time of administration of C_6 and atropine.

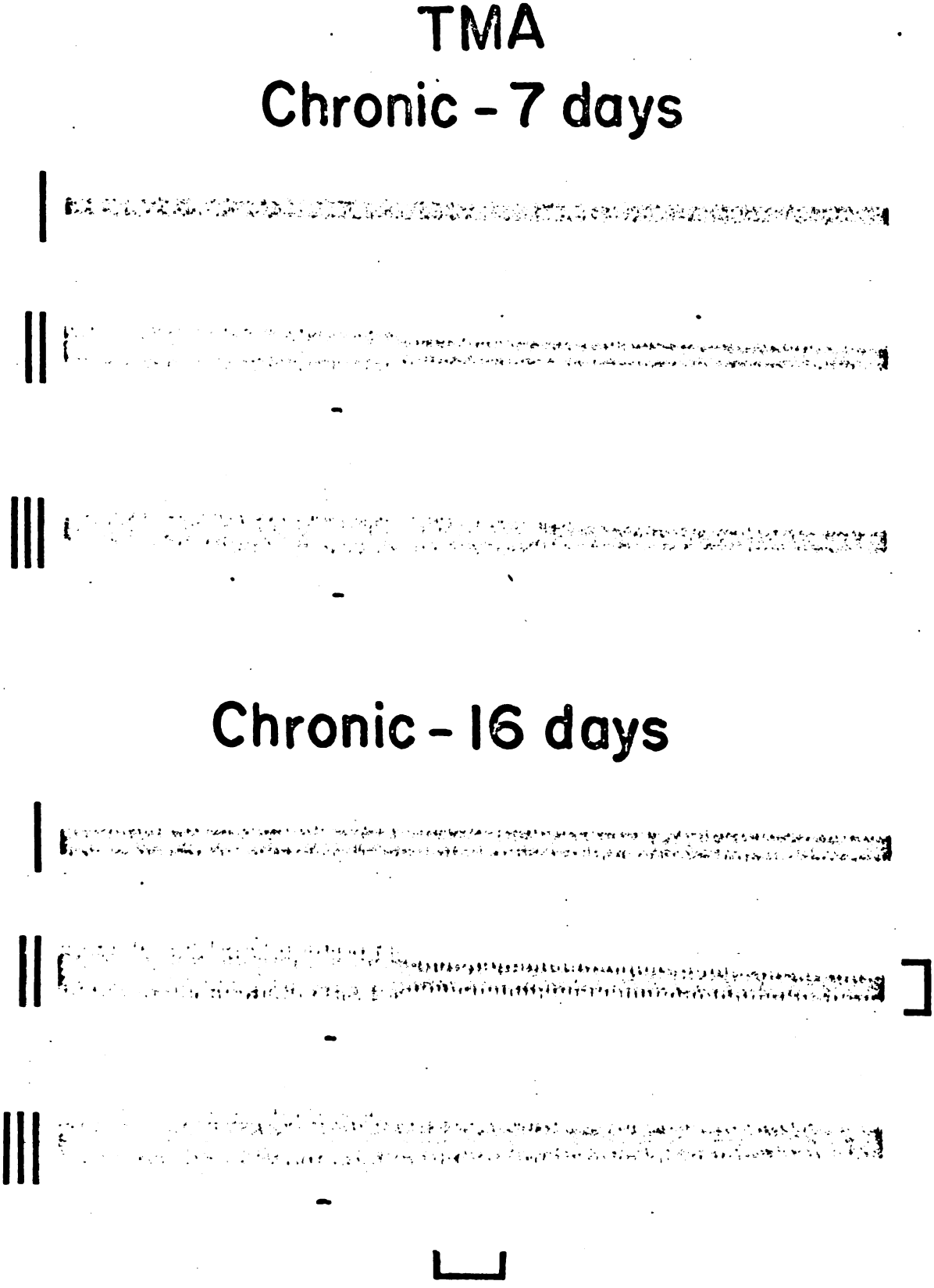

Figure 10. Postganglionic discharge evoked by infusion of TMA in ganglia denervated for 7 and 16 days.

atropine. As in the ganglia denervated for 7-11 days, a postganglionic action potential could not be elicited by stimulation of the remanents of the preganglionic nerve trunk rostal to the site of resection.

Two experiments were performed on cats where the ganglia had been chronically denervated for 53 and 54 days. In these experiments, a small compound postganglionic action potential was elicited during preganglionic stimulation of the residual sectioned nerve. Thus it appeared that partial reinervation had occurred. The blocking actions of atropine and C_6 on the postganglionic discharge evoked during the infusion of TMA was essentially the same as observed in the acutely decentralized ganglion. The discharge was blocked by either atropine or C_6.

DISCUSSION

It is surprising that the infusion of TMA evoked a postganglionic discharge that was markedly reduced or abolished by either C_6 or atropine. Yet TMA is usually considered as a nicotinic, ganglionic, stimulating agent (Volle, 1966) and its actions should therefore be similar to those of nicotine. However, the infusion of nicotine evoked a discharge that was blocked only by C_6 and unaffected by atropine. The question to be answered then, is "What possible mechanisms may be involved to explain the discharge evoked during the TMA infusion?"

The mechanisms involved in the initiation of the discharge evoked by TMA appeared to be of postganglionic origin. This was suggested by the fact that atropine and/or C_6 markedly reduced or abolished the postganglionic activity evoked by infused TMA in a ganglion which had been denervated for 7-11 days. The preganglionic terminals of the superior cervical ganglion disappear within this time after resection of the cervical sympathetic trunk (Koelle and Koelle, 1959).

One possible explanation of the action of both ganglionic blocking agents during the TMA infusion is that atropine was acting non-specifically at the C_6-sensitive site to block the discharge. However, from the following observations this does not appear to be the case.

1) Blockade of the discharge evoked during the infusion of TMA far outlasted the transient and partial block of

of transmission following the administration of atropine (see fig. 4). 2) Atropine blocked the postganglionic discharge without producing a concomitant repolarization of the ganglion (fig. 7). 3) The postganglionic discharge evoked by infused nicotine was unaltered by doses of atropine that abolished the discharge induced by infused TMA. 4) Atropine failed to alter the postganglionic activity evoked by single injections of TMA or nicotine.

Alternatively, the ability of both C_6 and atropine to block the discharge evoked during the infusion of TMA suggests that infusion of TMA initiated an interaction between the two excitatory ganglionic cholinoceptive sites. In this case, the interaction can be defined as the process whereby the action of TMA at one cholinoceptive site facilitates the initiation of action potentials at the second excitatory cholinergic receptor on the same ganglion cell. This, then, implies that the discharge of many of the individual cells participating in the recorded population response could be abolished by either atropine or C_6. The recent reports of Libet and Tosaka (1966, 1969) demonstrated the existence of both excitatory cholinoceptive sites on the same individual ganglion cells in the mammalian sympathetic ganglion.

At least three possibilities concerning the nature of the interaction between the two sites can be considered. 1) The weak muscarinic stimulating property of TMA may have been facilitated by the more familiar C_6-sensitive action

of the compound. Trendelenburg (1966a) has observed a weak muscarinic ganglionic stimulating property of TMA in the presence of nicotine-induced transmission block in the superior cervical ganglion while recording from the nictitating membrane. 2) Initiation of action potentials at the C_6-sensitive site could have been facilitated by the weak muscarinic stimulating property of the compound. 3) A combination of the above two possibilities may have occurred. In this case the postganglionic discharge would have emanated from both cholinoceptive sites.

No direct evidence was obtained to suggest that the initiation of asynchronous activity at the C_6-sensitive site by TMA was facilitated by an atropine-sensitive property of the drug. The administration of atropine failed to produce a consistent change in the demarcation potential of the ganglion even though it abolished the postganglionic discharge evoked during the TMA infusion. However, extracellular recording techniques may have limited the detection of atropine-induced repolarization of a few cells. In this regard, it should be noted that Libet (1964) observed that atropine at times prevented the gradual increase in the amplitude of the compound ganglionic action potential evoked by the first four or five volleys of a train of preganglionic stimuli.

The following observations suggest that the depolarization evoked at the C_6-sensitive site during the infusion of TMA facilitated the initiation of asynchronous action

potentials at the atropine-sensitive site. 1) Hexamethonium simultaneously repolarized the ganglion and blocked the asynchronous discharge evoked by infused TMA. 2) Atropine blocked the discharge evoked by the TMA infusion but failed to repolarize the ganglion. 3) A small atropine-sensitive discharge was observed in some animals following the administration of C_6. Thus it is probable that the weak muscarinic effect of TMA was greatly facilitated by the simultaneously occurring depolarization at the C_6-sensitive site in an unconditioned ganglion. The fact that atropine had no affect on the nicotine-induced discharge (Trendelenburg, 1966a) further suggests that the spread of depolarization from the C_6-sensitive site cannot alone initiate action potentials from the atropine-sensitive site, but must be accompanied by a concomitant direct activation or change in sensitivity of the atropine-sensitive site. This observation is also consistent with the lack of a ganglionic, muscarinic-stimulating property of nicotine.

Thus, it is proposed that the facilitatory interaction is the result of the depolarization initiated at the C_6-sensitive site which spreads via local current flow to the atropine-sensitive site to enhance the weak muscarinic stimulating action of TMA. Under this condition the postganglionic discharge would be emanating mainly from the atropine-sensitive site. Thus atropine blocks the postganglionic discharge directly at the site of initiation of the asynchronous discharge whereas C_6 blocks it

indirectly by eliminating the facilitatory spreading depolarization initiated at the C_6-sensitive site. The proposed facilitatory interaction is illustrated in fig. 11 in which E_1 and E_2 represent the C_6-sensitive site and the atropine-sensitive site respectively.

This proposal is supported by the reports of Trendelenburg (1966a, b) and is in agreement with the facilitatory action of nicotine described by Gebber (1968). While monitoring the movement of the nictitating membrane Trendelenburg (1966a, b) noted that ganglionic stimulation of angiotensin and other non-nicotinic agents was enhanced in the presence of nicotine. Hexamethonium blocked the facilitatory effects of nicotine on the ganglionic responses evoked by these agents. Gebber (1968) suggested that C_6-sensitive depolarization evoked by nicotine was responsible for the enhancement of non-nicotinic postganglionic discharges produced by ACh, MCh and serotonin. Blockade of depolarization by C_6 abolished the facilitatory effects of nicotine. Thus facilitation of the non-nicotinic discharge lasted only as long as ganglionic depolarization evoked by nicotine. It was proposed that the spread of depolarization from the C_6-sensitive site to the sites activated by non-nicotinic agents accounted for the facilitation (Gebber, 1968).

The reasons for the disparity between the ability of infusions and single doses of TMA to evoke atropine-sensitive firing are not clear. It is difficult to understand why a

Figure 11. Diagram of the proposed facilitatory interaction between the cholinoceptive sites.

The infusion of TMA initiates a depolarization at the C_6-sensitive site (E_1) which spreads via local current flow to the atropine-sensitive receptor (E_2). The placement and number of receptor sites is for convenience in illustrating the proposed theory. The exact location of the cholinoceptive sites on the ganglion cell membrane with respect to the axon hillock is unknown.

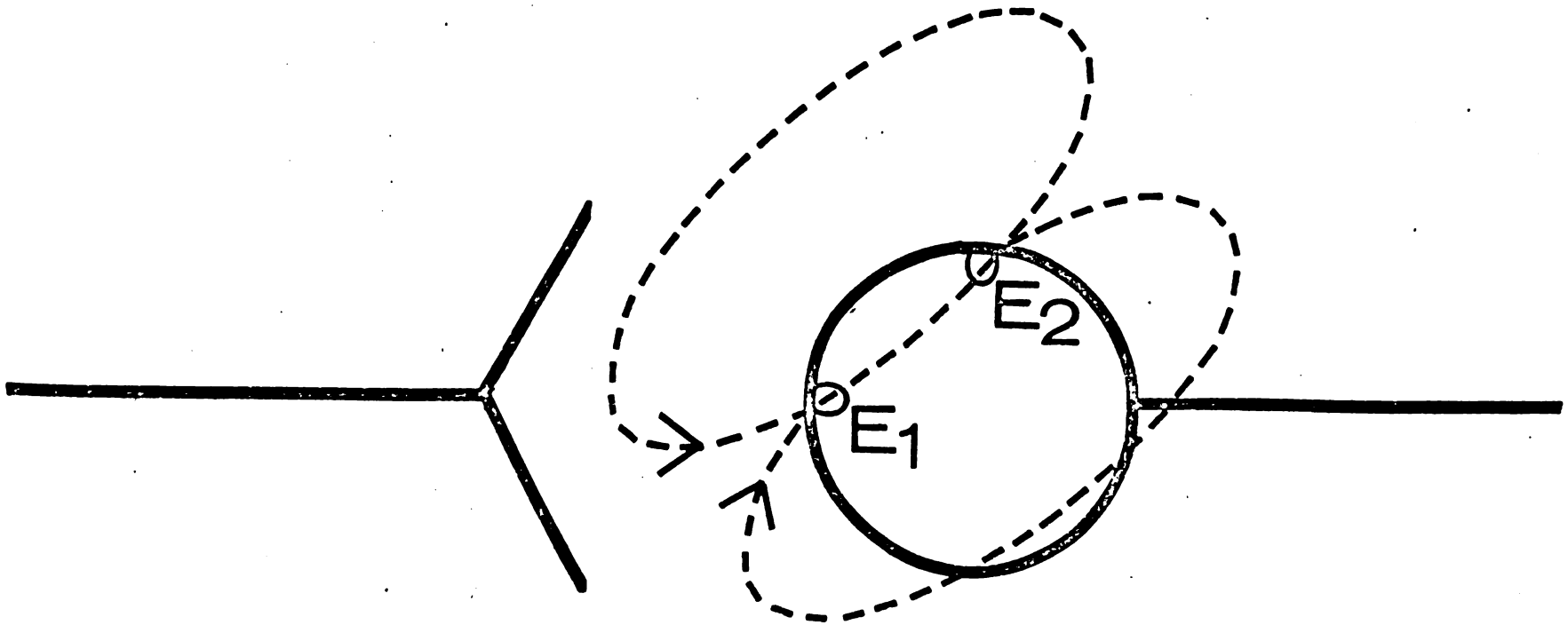

Figure 11. Diagram of the proposed facilitatory interaction between the cholinoceptive sites.

single injection of TMA which evoked C_6-sensitive depolarization of considerable amplitude and duration (Gebber and Volle, 1966) failed to evoke a discharge which was sensitive to atropine as well as C_6. Thus, other actions of TMA must have been involved in the initiation of the discharge which occurred during the infusion of TMA.

One such action may occur at the atropine-sensitive site. An infusion of TMA occasionally evoked a low amplitude atropine-sensitive discharge which was initiated 2-3 minutes following the administration of transmission blocking dose of C_6. Single doses of TMA failed to elicit a discharge that was sensitive to blockade by atropine. These observations indicate that the atropine-sensitive site on the ganglion was more sensitive to the direct stimulating actions of TMA when the compound was administered by constant infusion rather than by single injections. In ganglia conditioned with repetitive preganglionic stimulation the direct atropine-sensitive stimulating action of TMA occurred more frequently and was of greater amplitude. It should be stressed that in these experiments as well as in those of unconditioned ganglion the atropine-sensitive discharge was initiated after the administration of transmission blocking doses of C_6. In this case, the atropine-sensitive discharge was independent of any interaction between the two cholinoceptive sites. In contrast, repetitive preganglionic stimulation failed to unmask an atropine-sensitive discharge evoked by single injections of TMA. In view of these

observations, it appears that both the facilitatory effect of C_6-sensitive depolarization and some other mechanism of sensitization of the ganglion to the muscarinic stimulating properties of TMA were necessary for the initiation and maintenance of the largest component of the postganglionic firing observed in unconditioned ganglion during the infusion of TMA.

Equally puzzling is that, in contrast to the results obtained with single injections of TMA, infusion of TMA only occasionally evoked a discharge in unconditioned ganglia which were blocked by C_6 and resistant to atropine. The extremely small amplitude and infrequent occurrence of this component of firing is striking when one recalls that the infusion of TMA produced a C_6-sensitive depolarization whose amplitude was 25 to 50% of control spike height. Although increasing the infusion rate 5 to 10 times failed to increase the amplitude of the atropine-resistant discharge in control ganglia, this component of firing was considerably larger in ganglia which had been denervated for 16-23 days and in those which had been conditioned with a preganglionic tetanus.

One possible explanation for the small and infrequent pure C_6-sensitive discharge (atropine-resistant) evoked during the infusion of TMA is that the threshold of depolarization (Eccles, 1964) for the C_6-sensitive site was not obtained in most ganglion cells during the infusion. This however, does not appear to be the case since the amplitude

of the C_6-sensitive depolarization (25 to 50% of control spike height) evoked during the TMA infusion was comparable to that evoked by single injections of TMA which produced good C_6-sensitive firing (Takeshige and Volle, 1964; Gebber and Volle, 1966). The threshold of depolarization may play an important role in the mechanism if accommodation of the C_6-sensitive site had occurred (see below).

The second explanation may deal with the difference in the rate of depolarization following the administration of TMA. Sasaki and Otani (1961) have reported that the threshold of depolarization of the cat motoneuron may vary according to the different time course of augmenting depolarization. The slower the rate of depolarization (cathodal current) the higher the threshold of depolarization. A similar mechanism may have been involved during the infusion of TMA. The C_6-sensitive site may discharge only in the face of a rapid depolarization provided the threshold of spike initiation is reached. Gebber and Volle (1966) have demonstrated a sudden depolarization of the ganglion and a simultaneous C_6-sensitive postganglionic discharge following an intraarterial injection of TMA. A gradual depolarization of the C_6-sensitive site during the infusion of TMA to a level equivalent to that following a single injection of TMA might not produce equivalent C_6-sensitive responses. A rapid upward shift in the demarcation potential tracing (denoting depolarization) was never observed following the initiation of the TMA infusion. It was not possible to

accurately follow changes in the demarcation potential of the ganglion for periods longer than three minutes. Therefore it was assumed that the infusion of TMA evoked a depolarization which gradually reached peak amplitude. Thus, during the infusion of TMA, the C_6-sensitive site may have accommodated to the gradual depolarization and thereby raised the threshold of depolarization for spike initiation which limited the pure C_6-sensitive response. However, in the presence of the depolarization induced by infused TMA, transmission was only slightly altered yet there was very little pure C_6-sensitive firing. This disparity may also be explained in terms of the rate of depolarization. Rapid C_6-sensitive depolarization evoked by neurogenically released ACh, (Eccles, 1963) initiated the postganglionic action potential. In this case, the rapid change in ganglionic depolarization would be the major factor in evoking the C_6-sensitive postganglionic spike.

However, the possibility exists that the infused TMA was acting at C_6-sensitive sites other than those involved in transmission. This was suggested by the fact that transmission was only slightly affected when the TMA infusion evoked a large C_6-sensitive depolarization and an infrequent, low amplitude atropine-resistant discharge. This is in agreement with the reports of Riker (1967) and Gebber (1968) which demonstrated that the subsynaptic C_6-sensitive sites involved in transmission may differ from the C_6-sensitive sites activated by nicotine like drugs.

The ganglionic atropine-sensitive stimulating action of ACh was also prominent when that compound was infused rather than injected in a single dose. The infusion of ACh usually evoked a postganglionic discharge which was sensitive to blockade by atropine and unaffected by C_6. In contrast, a single injection of ACh evoked a C_6-sensitive discharge in an unconditioned ganglion. Takeshige and Volle (1962) reported that the threshold dose of a single injection of ACh required to activate the atropine-sensitive 'late' response was much higher than that required for activation of the C_6-sensitive site. It is interesting that the changes in the reactivity of the two cholinoceptive sites observed during the infusion of ACh were similar to those observed for single injection after repetitive preganglionic stimulation and the administration of anticholinesterase agents. As noted by Takeshige and Volle (1962) following these conditioning procedures, the threshold for activation of the two cholinoceptive sites by a single injection of ACh was reversed. Activation of the 'late' atropine-sensitive discharge required smaller doses of ACh than activation of the 'early' C_6-sensitive response.

The prominence of the atropine-sensitive discharge elicited during the infusion of ACh may also be explained as the result of the gradual rate of depolarization of the C_6-sensitive site. Sasaki and Otani (1961) reported that due to the accommodation of the initial segment of the cat motoneuron the site of spike initiation changed. Their

results indicated that the spikes are generated from the initial segment when a rectangular current (rapid depolarization) is applied and from the soma when the current rise is slow enough. Similarly, the C_6-sensitive site may have accommodated to the gradual depolarization during the infusion of ACh. This may have raised the threshold of the C_6-sensitive site above that of the atropine-sensitive site so that the initiation of asynchronous action potentials was at the atropine-sensitive site.

SUMMARY

It has been shown that altering the mode of administration of TMA changed the pharmacologic properties of the postganglionic discharge evoked by TMA. Single intra-arterial injections of TMA evoked a brief burst of activity that was blocked by C_6 and unaffected by atropine. In contrast, a constant infusion of TMA evoked a continuous postganglionic discharge that was sensitive to blockade by either C_6 or atropine.

The results with infusions are explained on the basis of a facilitatory interaction between the two pharmacologically distinct cholinoceptive sites. It is proposed that constant infusion of TMA evoked a depolarization at the C_6-sensitive site which spread via local current flow to the atropine-sensitive site to enhance the responsiveness of the muscarinic receptor. Thus, the weak muscarinic stimulating properties of TMA are greatly facilitated during the infusion of TMA. The spread of depolarization to the atropine-sensitive site and the concomitant direct muscarinic stimulating action of TMA are necessary for the facilitatory interaction. The actions of the ganglionic blocking agents can be explained in the following way: Atropine blocked the postganglionic discharge evoked by TMA directly at the site of initiation. C_6 blocked the discharge indirectly by abolishing the spreading facilitatory depolarization.

This proposal assumed that the two excitatory cholinoceptive sites are located on the same individual ganglion cell. The recent studies of Libet and Tosaka (1966, 1969) indicate that this assumption can be made.

Similarly, this study showed that the mode of administration of ACh is a critical factor as concerns the relative participation of the two cholinoceptive sites in the discharge initiated by this compound. Constant infusion of ACh usually evoked an atropine-sensitive discharge. In contrast Takeshige and Volle (1962) have reported only a C_6-sensitive discharge in unconditioned ganglion following single intraarterial injections of ACh. It appeared that the threshold of activation of the two cholinoceptive sites were reversed during the infusion of ACh. This could also be explained as facilitatory interaction between the cholinoceptive sites together with a possible accommodation of the C_6-sensitive site to the gradual depolarization evoked by the ACh infusion.

The role of the proposed facilitatory interaction in the normal integrative functions of sympathetic ganglia is yet to be determined.

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