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THE UNIVERSITY OF OKLAHOMA
GRADUATE COLLEGE

ANALYSIS OF MUSCARINIC TRANSMISSION IN THE SUPERIOR CERVICAL AND CILIARY GANGLION OF THE CAT

A DISSERTATION
SUBMITTED TO THE GRADUATE FACULTY
in partial fulfillment of the requirements for the
degree of
DOCTOR OF PHILOSOPHY

BY
JOSEPH ALOIS RIEGER
Norman, Oklahoma
1975

ANALYSIS OF MUSCARINIC TRANSMISSION IN THE SUPERIOR CERVICAL AND CILIARY GANGLION OF THE CAT

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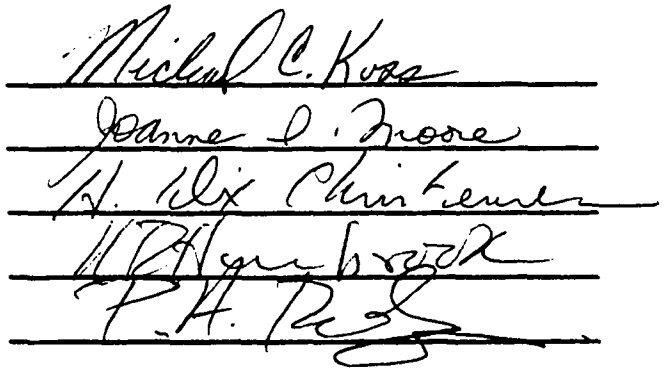

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CHAPTER I

IMPULSE TRANSMISSION IN AUTONOMIC GANGLIA

HISTORICAL INTRODUCTION

The early work of Dale (1914) brought forth the terms nicotinic and muscarinic which are still in use today. These words were introduced when little knowledge was available concerning the role played by acetylcholine (Ach) in autonomic ganglionic transmission. The genesis of these terms arose from observations made on changes in blood pressure following the injection of variable quantities of Ach, other choline esters, nicotine, and muscarine into intact animals. Muscarinic action was defined by Dale as "the action which true muscarine exhibits in its pure form, uncomplicated by the nicotinic action". He assumed that the muscarinic effects of choline esters were "purely peripheral in their origin, unaffected by nicotine in large doses, but rapidly abolished by small doses of atropine". On the other hand, the term nicotinic referred to the action of nicotine, Ach, and other cholinomimetic agents on all autonomic ganglia, as well as the neuromuscular junction. Since the peripheral cholinergic receptors are responsive to smaller doses of Ach and similar

compounds than are those in autonomic ganglia, atropine was frequently used in studies involving cholinergic agents on autonomic ganglia for many years following Dale's observations. However, as early as 1932, Koppanyi called attention to the possibility of muscarinic receptors in sympathetic autonomic ganglia and the possibility of masking them with atropine.

During the nineteen fifties and sixties numerous publications have appeared in relation to various aspects of impulse transmission in autonomic ganglia. A frequently used experimental preparation has been the superior cervical ganglion (SCG) of the cat or dog. Paton and Perry (1953) used this preparation to show that the ganglionic blocking agents could be placed in one of two categories, depolarization and non-depolarization blockade. The first would include those agents that block transmission following depolarization of the post synaptic membrane such as Ach, nicotine, or tetramethylammonium (TMA) and the second by agents that block without depolarizing the membrane or by competing with Ach. Tetraethylammonium (TEA), hexamethonium (C-6), or d-Tubocurarine (d-TC) are examples of agents which fall into the latter category. These authors pointed out that nicotine has a dual effect, blocking initially by depolarization and subsequently by a nondepolarizing or competitive action. Eccles and Libet (1961) studied ganglionic transmission in curarized SCG and noted that atropine in low concentrations could abolish the late negative and strongly depress the positive potential, with little effect on the negative potential or the primary synaptic potential. Takeshige and Volle (1962) observed a bimodal response to injected Ach following either pretreatment of the SCG of cats with physostigmine or repetitive

preganglionic stimulation. The early response was blocked by d-TC, but not by small doses of atropine. Conversely, the late response was sensitive to blockade by atropine, but not large doses of d-TC. Physostigmine, administered in relatively large doses, evoked a postganglionic discharge which was blocked by atropine, but not d-TC or mecamylamine. Furthermore, repetitive stimulation of ganglia treated with large doses of d-TC produced an asynchronous postganglionic discharge which was sensitive to blockade by atropine.

Attempts to distinguish between nicotinic and muscarinic receptors in autonomic ganglia have been carried out by using specific agonists. In 1951, Chen et al. published a paper concerning the actions of the now widely used nicotinic stimulant 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP). They noted it effected contraction of the nicotinic membrane of the cat, elevated the blood pressure, and resulted in an increase in urinary bladder pressure in the dog. These effects could be prevented by previous administration of TEA or C-6. Ling (1959) studied the blocking action of DMPP, and demonstrated that it blocked peristalsis in isolated guinea pig ileum which had been stimulated by raising intraluminal pressure. He also showed that it inhibited the response of the rat diaphragm and cat gastrocnemius following stimulation of their motor nerves. Ambache et al. (1956) studied the effect of muscarine on the perfused SCG of the cat, eliciting a contraction of the nictitating membrane in 14 out of 16 preparations. Although atropine administered intravenously (i.v.) did not antagonize this response, a small dose (1 microgram) added to the perfusate and thus directly to the ganglion did effect muscarine antagonism.

In 1961, Roszkowski aroused considerable excitement following publication of data relating to pharmacological properties of 4-(m-chlorophenylcarbamoyloxy)-2-butynyltrimethyl-ammonium chloride (McN-A-343), which he asserted acted selectively on sympathetic autonomic ganglia. Intravenous injection of this substance into anesthetized dogs or cats caused an initial transient depressor response followed by a more marked and sustained pressor effect. He demonstrated that atropine (1 mg/kg i.v.) completely blocked both components of the diphasic response, whereas it had no effect on the pressor response elicited by DMPP injected i.v.. The atropine blockade persisted for a period greater than six hours and the administration of greater quantities of McN-A-343 did not reverse the blockade. However, it was shown that microgram doses of atropine were sufficient to block pressor responses elicited by small doses of McN-A-343 and this type of blockade could be reversed by greater quantities of McN-A-343 in 30 to 45 minutes. Roszkowski also demonstrated that C-6 does not block the pressor response elicited by McN-A-343; in fact, in some instances it augments it. In reserpine pretreated dogs, the response to McN-A-343 was predominately depressor with the pressor component almost completely obliterated except when high doses (200 µg/kg i.v. or more) were used.

Franko et al. (1963) reported that N-benzyl-3-pyrrolidyl acetate methobromide (AHR-602), possessed similar pharmacological properties to McN-A-343. Intravenous administration of 0.1 to 10 mg/kg usually produced a biphasic response, an initial brief depressor response followed by a more prolonged and pronounced pressor response. The pressor response, presumably a consequence of activation of receptors in sympathetic ganglia, was enhanced by competitive type ganglion blocking agents

and was susceptable to atropine antagonism. Like McN-A-343, it markedly stimulated salivary secretions.

Jones (1963) carried out an extensive study of the effects of muscarine, McN-A-343, and AHR-602 on the SCG of cats in the absence of atropine and measured contractions of the nictitating membrane (NM). Small doses administered via the lingual artery so as to go directly to the SCG (Trendelenburg, 1956) elicited responses which were ineffective when injected during the depolarization phase of ganglionic blockade produced by TMA or nicotine. During the non-depolarizing phase of blockade, all three agents were able to stimulate the ganglion at a time when DMPP and nicotine produced no response. Consistently, the ganglionic response to these three agents was not blocked by C-6, but was antagonized by small amounts of atropine. Cocaine, morphine, and methadone were shown to reduce the response of the ganglion to these agents, while being ineffective against nicotine, DMPP, KCl, and preganglionic stimulation. Subthreshold amounts of McN-A-343, AHR-602, and muscarine were found to facilitate submaximal preganglionic stimulation. Supramaximal preganglionic stimulation often potentiated the response of the ganglia to these three substances.

The observations of Sanghvi et al. (1963), who also used the SCG of the cat and intra-arterial (i.a.) injections to the ganglion, included a rapid and brief response with DMPP, contrasted with a less rapid and more sustained contraction of the NM with muscarine. In addition, subthreshold doses of muscarine were shown to potentiate both the contraction of the NM as well as postganglionic action potential (both the S-1 and S-2 components) evoked by preganglionic stimulation. Trendelenburg (1966a)

used this same experimental preparation to demonstrate two procedures which effect facilitation of ganglionic responses to certain non-nicotinic stimulants. First, a preceding period of preganglionic stimulation (5 seconds) was shown to augment the ganglionic stimulant properties of angiotensin and bradykinin, as well as histamine and McN-A-343. Second, repeated injections of nicotine which produces a late non-depolarizing type of block, was shown to enhance the action of angiotensin but reduce that of bradykinin. Subsequent injections of C-6 restored ganglionic responses of both polypeptides to normal. In a concurrent publication, Trendelenburg (1966b) demonstrated conclusively that ganglionic transmission can occur in the SCG via a muscarinic mechanism under certain conditions. During the late non-depolarizing phase of nicotine block, the ganglion responded to non-nicotinic or muscarinic agents at a time when there was an irreversible type of antagonism to nicotinic stimulants. Also, during the late phase of nicotine block, transmission occurred following preganglionic stimulation.

Several investigators have used the inferior mesenteric ganglion (IMG) of the cat to study the mechanisms involved in ganglionic transmission. Herr and Gyermek (1960) recorded the frequency of nerve action potentials in isolated postganglionic nerve fibers following i.a. injection of drugs close to the ganglion. As expected, DMPP caused a transient increase in the frequency of firing. Muscarine failed to increase the rate of firing in these experiments; however, atropine (1 mg/kg i.v.) had been given earlier. Subsequently, Gyermek et al. (1963) clearly demonstrated the ganglionic stimulant properties of muscarine on the IMG of the cat in the absence of atropine by recording postganglionic potentials.

They noted its effect was slow in onset, relatively long in duration, and highly sensitive to atropine. Gyermek and Bindler (1962) observed that 5-HT likewise had stimulant action on the IMG, but apparently activated different receptors than those responsive to DMPP and muscarine. Morphine and C-6 were shown to be effective antagonists to the actions of 5-HT and DMPP, respectively. Murayama and Unna (1963), who also recorded postganglionic potentials from the IMG of the cat, demonstrated a temporal difference in the response elicited by i.a. injections of McN-A-343, pilocarpine and DMPP. The increased discharge rate lasted for several minutes with McN-A-343 and less than one minute with DMPP. Atropine was very effective in blocking the effects of the former and pilocarpine whereas C-6 was effective in blocking only DMPP.

The stellate ganglion of dogs and cats has been utilized more recently to study impulse transmission in autonomic ganglia. Brown (1967) demonstrated that stimulation of the thoracic nerve trunk increased mean arterial blood pressure and heart rate which persisted, although in most cases the magnitude was reduced, during C-6 infusion or after local application of a 0.25 percent solution of nicotine to the ganglion. In 40 out of 46 experiments, the persistent blood pressure and heart rate response was abolished by i.v. injection of atropine sulphate in doses ranging from 10 to 100 μg/kg. When the response to preganglionic stimulation was abolished by atropine, stimulation of the postganglionic trunks evoked a rise in blood pressure and heart rate identical with that elicited before administration of the drug. Flacke and Gillis (1968) demonstrated convincingly that transmission via nicotinic or muscarinic pathways is possible in the stellate ganglia of the dog. Curves relating stimulation frequency to heart rate increases were only shifted to the right by large

doses of non-depolarizing or nicotinic blocking agents. Maximal responses to nerve stimulation were not affected. The administration of 30 μg/kg of atropine after C-6 virtually blocked the effect of preganglionic stimulation. Curves obtained by postganglionic stimulation during the period of complete block were very similar to control values. Atropine alone, even in large doses, did not alter the frequency response curve to preganglionic stimulation in the absence of C-6. These same investigators (Gillis et al., 1968) studied the effect of physostigmine and DFP on transmission in this system. The anticholinesterase agents did not potentiate transmission during muscarinic blockade with submaximal stimulation or even when partial block by C-6 or mecamylamine was produced. In contrast, the same doses of anticholinesterase agents shifted the frequency response curve to the left when transmission was muscarinic. By cannulating the brachial artery of the dog with a small polyethylene catheter and advancing it proximally until the tip was approximately at the junction of the right mammary and right subclavian arteries, Flacke and Fleisch (1970) were able to demonstrate the cardio-accelerator effects of small doses of ganglionic agonists. DMPP and McN-A-343, in doses of 80 and 200 μg respectively, caused a marked increase in heart rate, although the time course was noticeably different.

Smith (1966a) observed a species difference in the pressor response elicited by McN-A-343 in vagotomized preparations. The cat and rat were much more responsive than the guinea pig or rabbit to i.v. injections. An interesting observation was that physostigmine antagonized the pressor effect of McN-A-343 without affecting the depressor phase. Procaine, neostigmine, DMPP, diphenhydramine and pronethalol were reported

to act in a similar fashion. In contrast, none of the aforementioned antagonists affected DMPP induced pressor responses. Contrasting effects of McN-A-343 and DMPP were observed in vivo on the intestine of a decapitate cat following i.v. injections. With McN-A-343 a prolonged inhibition was usually observed, whereas with DMPP a triphasic pattern was seen; (an initial rapid but brief spasm or increase in tonus, an inhibition, and then an increase in motility). His data also indicates that the rat ileum in vitro is responsive to McN-A-343, but the potency of McN-A-343 is only about one thousandth that of methacholine. In another report, Smith (1966b) noted that the ganglionic stimulant properties of 5-HT and histamine could also be antagonized by the aforementioned antagonists of McN-A-343. Raising the extracellular concentration of calcium ions in the SCG was also found to have a similar selective depressant effect on responses to non-nicotinic as opposed to nicotinic stimulants. Lowering of the extracellular concentration of calcium ions had the opposite effect. Bentley (1972) studied the effect of nicotine and McN-A-343 on the hypogastric ganglion of the guinea pig and rat. The perfused and isolated ganglion of the guinea pig responded repeatedly to nicotinic stimulants, whereas that of the rat did not. Neither species responded well to non-nicotinic stimulants. Vanov (1965) reported that an i.v. dose of 10 μg of McN-A-343 in the rat increased blood pressure without affecting the tonus of the bladder.

Hilton and Steinberg (1966) elevated the intracranial fluid pressure in conjunction with major vessel occlusion as a means of studying ganglionic transmission. The administration of 1.0 mg/kg of chlorisondamine reduced the amount of vasoconstriction elicited by this physiological

method, but did not totally block the response. Subsequent administration of atropine produced almost total blockade. Fenner and Hilton (1963) had previously shown that neostigmine could restore the carotid occlusion reflex which had been blocked by chlorisodamine or C-6. The restoration was sensitive to blockade by atropine.

In retrospect, a great many investigations have been carried out with regard to impulse transmission in autonomic ganglia. Most of them have dealt with transmission in the sympathetic division of the autonomic nervous system (ANS). Sufficient evidence exists to accept the hypothesis that transmission can occur via nicotinic or muscarinic pathways in several ganglia. In addition, there may be other non-cholinergic ganglionic receptors responsive to such agonists as 5-HT, angiotensin, bradykinin, and histamine. It also is apparent that comparatively little work has been done on transmission in the parasympathetic division of the ANS. This is understandable in lieu of the anatomical and physiological differences between the two systems. There are only three anatomically distinct ganglia in the parasympathetic system-the ciliary, otic, and the sphenopalatine. The ciliary, at least in the cat, does afford one the opportunity of stimulating preganglionically and recording potentials postganglionically. Parasympathetic ganglia lying within the heart, intestines, or bladder have far too short postganglionic fibers to permit recording of changes in potentials. The existence of a dual cholinergic mechanism in the two junctions (ganglionic and neuroeffector) further complicates the problem of studying impulse transmission in this division of the ANS.

Nevertheless, several attempts have been made to investigate

transmission in parasympathetic ganglia. Some forty years ago, Henderson and Roepke (1934) isolated parasympathetic ganglia in the bladder of the dog, and judged they lay approximately 0.5 cm. from the bladder. They reported blockade of transmission following application of pledgets of cotton moistened with a one per cent solution of nicotine. Similarly, isolation of the ciliary ganglion, and stimulation of the preganglionic nerve distal to where it was severed or application of pledgets of cotton moistened with a 5 per cent solution of Ach to the ganglion effected good pupillary constriction. In 1936, Christensen, an anatomist, reported that the ciliary ganglion of the cat has no sympathetic fibers passing through it and therefore the short ciliary nerves when they first arise from the ciliary ganglion contain only postganglionic parasympathetic fibers. A year later (1937), Whitteridge studied transmission in this ganglion and noted that action potentials recorded from the short ciliary nerves following stimulation of the intracranial portion of the third cranial nerve were abolished following the painting of the ganglion with nicotine. He described the normal action potentials of the ciliary ganglion as diphasic, consisting of a rapid negative spike lasting 5 to 10 milliseconds, followed by a slow positive wave which lasted 125 to 150 milliseconds. He also observed smaller secondary negative potentials which followed the initial negative spike potential. Luco and Marconi (1949) stimulated the ciliary and SCG preganglionically simultaneously, but on opposite sides while observing the blocking effects of i.v. administered TEA. They reported incomplete blockade of ciliary ganglionic transmission at a dose which completely blocked SCG transmission.

In 1953, Perry and Talesnick reported results of a rather extensive study concerning the role of Ach on transmission in the ciliary ganglion of cats. Drugs were injected in a retrograde fashion into the lingual artery and it was noted that Ach was without effect unless large doses, (0.2 to 1.0 mg) were given. Ach was shown to depolarize the cells of the ganglion, accompanied by a partial block of the action potential spike and by a characteristic change in the shape of the action potential complex. In one experiment it was shown that 20 μg of Ach produced a potentiation of the postganglionic spike potential in response to submaximal preganglionic stimulation. C-6, 250 μg i.a., produced a block of 30 per cent of the spike height without any depolarization, and in the absence of the striking change in shape that was characteristic of both Ach and nicotine block. In experiments in which pupil size was recorded it was found that C-6, in i.v. doses of 5 mg/kg, would produce complete block of preganglionic stimulation without in any way affecting postganglionic stimulation. In other experiments nicotine was given i.a. in doses of 250 μg and its main effect was a constriction of the pupil, which thereafter did not respond to preganglionic stimulation, although postganglionic stimulation was still effective. (This constriction was followed by a dilation which was abolished by removal of the adrenal glands). After the short ciliary nerve had been cut, nicotine had no effect on the pupil. Although the significance is not clear, it was noted that the whole action potential complex of the ciliary ganglion lasts only 150 milliseconds and is thus much shorter than in the SCG which lasts 600 to 900 milliseconds. Otherwise, the shape of the complex is similar, although the after-positivity is relatively smaller.

Nisida and Okada (1960) recorded nerve action potentials from the short ciliary nerve of the cat while studying the activity of the pupillo-constrictor centers. They reported that action potentials from the short ciliary nerve bundle consist of spikes of various magnitudes, but those from a single active unit or nerve fiber consist of a series of impulses of the same amplitude, recurring at regular intervals. The impulse frequency from the individual fiber varies with each animal and even in the same cat, although the range is approximately 4 to 10 per second. Action potentials from the short ciliary nerve were completely abolished following severance of the homolateral oculomotor nerve in the cranial cavity. These authors also recorded action potentials from the oculomotor and Edinger-Westphal nuclei in the midbrain. In experiments whereby light of increasing intensity was applied to the contralateral eye, they found action potentials recorded from short ciliary nerves also increased in frequency. The threshold stimulus was usually about 30 lux. When the light stimulus was repeatedly applied at short intervals, e.g. less than 5 or 10 minutes, the response decreased. In studying the relationship between discharge pattern and intensity of light stimulus they noted that weak to moderate light resulted in an on-reponse without an after discharge, whereas a strong stimulus effected an on-response with an after discharge.

In 1960, Alonso-deforida et al. demonstrated that continuous infusion of a variety of ganglionic blocking agents including TEA, C-6, d-TC, diphenmethamil, methantheline, and methylscopolamine resulted in a 100 per cent reduction in the amplitude of ciliary postganglionic action potentials following supramaximal preganglionic stimulation. They also

noted that at low dose levels, most of the blocking agents tested produced facilitation at the ciliary ganglion.

Flacke and Gillis (1968) used the heart lung preparation of the dog to study transmission in parasympathetic ganglia. A good dose response relationship, i.e. increased bradycardia, was observed following stimulation of the preganglionic nerves with supramaximal stimulation of increasing frequencies. Administration of C-6 in doses between 4 and 10 mg blocked this effect completely. However, a small amount of physostigmine, injected after the blocking doses of C-6, induced a fall in heart rate and a partial return of the response to vagal stimulation, which was susceptable to blockade by very small doses of atropine. Although their results seemed to support the concept of muscarinic receptors in autonomic ganglia, they readily admitted that the proof was not unequivocal. In 1971, Chiba et al. reported that McN-A-343 injected into the sinus node artery of dogs vagotomized at the mid-cervical level blocked sinus bradycardia induced by electrical stimulation of the right vagus, without blocking the bradycardia induced by administration of Ach or nicotine into the sinus node artery. McN-A-343, given alone in doses of 1 to 10 μg, effected a modest bradycardia, which could be blocked by prior administration of atropine.

In a very brief report Saxena (1971) reported that in the dog,

> Both McN-A-343 (25 to 200 μg) and AHR-602 (0.1 to 1.0 μg), when injected i.a. to the urinary bladder of the dog caused a dose-dependent contraction of the organ. The stimulant action of the above drugs was blocked not only by a low dose of atropine (5 μg/kg), but also during depolarizing ganglionic blockade induced by nicotine, TMA, or DMPP. The administration of guanethidine, C-6, and nicotine (in amounts that caused non-depolarizing block of the ganglia), cocaine and morphine did not appreciably modify the response to the ganglionic stimulants.

No quantitative data was shown to support this statement. Taira et al. (1971) also used the canine urinary bladder in an attempt to demonstrate the presence of muscarinic receptors within intramural parasympathetic ganglia. Their most convincing data show a tetrodotoxin blockade of increased intra-bladder pressure elicited by i.a. injection of McN-A-343. Complete blockade was effected only when a monophasic response was elicited. During a diphasic response, only the first component was blocked. Tetrodotoxin readily and completely blocked DMPP induced intra-bladder pressure. Chiba et al. (1971) reported they were unable to see a tetrodotoxin blockade of McN-A-343 induced bradycardia. The observations of Taira et al. are clouded by the complication that repeatedly arises when one is looking at end organ responses in a system containing two cholinergic synapses.

Figure 1 is a schematic representation of the mammalian brain showing diagramatically the main pathways involved in dilation and constriction of the pupil and contraction of the NM. The solid line represents the efferent sympathetic pathway via the SCG to the pupil and NM. The dashed line represents the efferent parasympathetic pathway via the 3rd cranial nerve (C.N.) and ciliary ganglia to the sphincter of the iris. The dotted lines represent the inhibitory pathways to the oculomotor nucleus.

The aim of this investigation was to study impulse transmission in the SCG and ciliary ganglion, with particular emphasis on muscarinic mechanisms. The i.a. administration of selectively acting ganglionic agonists and antagonists was utilized in testing for the presence of muscarinic receptors in these ganglia. In addition, frequency-response

curves were utilized in gaining a quantitative assessment of impulse transmission via nicotinic pathways, or during the presence of non-depolarizing nicotinic receptor blockade, via muscarinic pathways.

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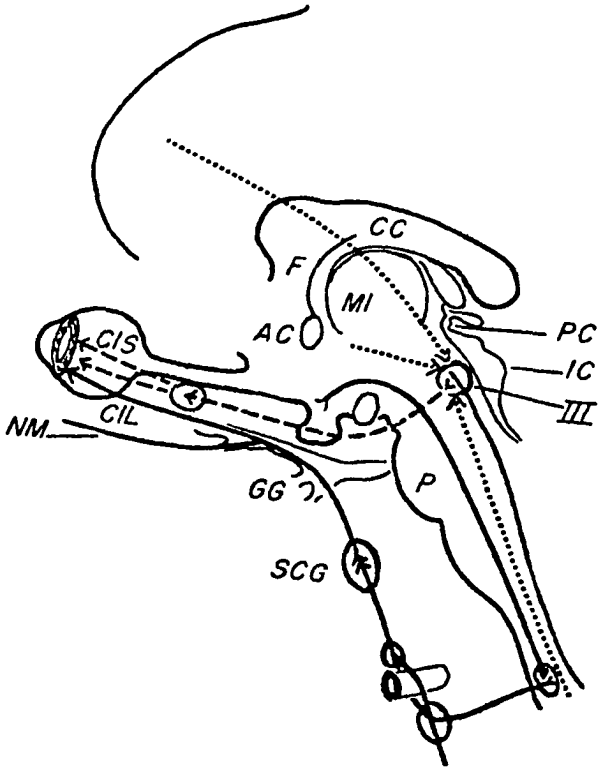

Figure 1. AC - anterior commissure, CC - corpus callosum, CIL - Long ciliary nerves, CIS - short ciliary nerves, F - fornix, GG - gasserian ganglion, IC - inferior colliculus, MI - massa intermedia, NM - nictitating membrane, P - pons, PC - posterior commissure, SCG - superior cervical ganglion, III - oculomotor nucleus. (From Loewenfeld, Doc. Ophthal., 1958, 12: 191-448).

CHAPTER II

MUSCARINIC MECHANISMS IN THE SUPERIOR CERVICAL GANGLION OF THE CAT

ABSTRACT

Muscarinic mechanisms within the superior cervical ganglion (SCG) eliciting contraction of the nictitating membrane (NM) and dilation of the pupil were studied in cats anesthetized with alpha-chloralose. Frequency-response curves were obtained by unilateral stimulation of the preganglionic sympathetic nerve. Sympathetic responses of the NM and pupil were recorded simultaneously in the same preparations. Differences in the curves representing responses of the two effector organs were observed. The threshold of activation was higher and the slope of the frequency-response curve was steeper for the pupil than for the NM. Hexamethonium (C-6) was more effective in reducing pupillary than NM responses. Subsequent administration of atropine further reduced those responses remaining in both effectors following nicotinic blockade. Selective activation of the NM with respect to the pupil was observed following the i.a. administration of 30 to 40 μg of McN-A-343. This observation, plus the previous one that C-6 was less effective in blocking NM compared to pupillary responses elicited by electrical stimulation of the preganglionic nerve, suggests that there may be quantitative

differences in the degree of muscarinic transmission within the SCG depending upon which effector is observed.

INTRODUCTION

The SCG of the cat has long been utilized in studying mechanisms involved in autonomic ganglionic transmission. The observations of Dale (1914) contributed greatly to the concept of nicotinic like receptors being singularly present in all ganglia, and muscarinic like receptors being singularly present in all parasympathetic neuroeffector junctions. Koppanyi (1932) questioned this rather simplistic concept and suggested that muscarinic receptors might also be present in autonomic ganglia. Ambache et al. (1956) studied the effects of muscarine, and Jones (1963) investigated the effects of muscarine, McN-A-343, and AHR-602 on the SCG of the cat. They demonstrated that these agents administered i.a., close to the ganglion, were capable of evoking a contraction of the NM. Atropine, administered in very small doses, totally abolished these muscarinic responses. Flacke and Fleisch (1970) carried out extensive studies regarding the effects of ganglionic agonists (including DMPP and McN-A-343) and antagonists (including C-6 and atropine) on cardiac sympathetic ganglia of the dog.

Several investigators have utilized electrophysiological techniques in studying actions of these agents on sympathetic ganglia. Sanghvi et al. (1963) recorded increased activity in postganglionic sympathetic nerve fibers following i.a. administration of DMPP and McN-A-343 to the SCG. Murayama and Unna (1963) were able to demonstrate an

increase in postganglionic potentials recorded from the hypogastric nerve fibers following i.a. administration of DMPP and McN-A-343 to the inferior mesenteric ganglion of cats.

Quantitative measurements of impulse transmission via nicotinic and muscarinic pathways have been made in more recent investigations. Flacke and Gillis (1968) channeled impulse traffic through specific pathways within the stellate ganglion by using selectively acting ganglionic blocking agents, i.e. non-depolarizing nicotinic blocking agents such as C-6 and muscarinic blocking agents such as atropine. Frequency-response curves, obtained by stimulating the preganglionic nerve trunk in the presence of large doses of one of the ganglionic blocking agents, were used to estimate the degree of impulse transmission via one or another pathway. The curves relating stimulation frequency to heart rate increases were only shifted to the right in the presence of supramaximal doses of C-6. The same maximal increase in heart rate was seen before and after nicotinic blockade. Atropine, 30 μg/kg i.v., administered after C-6, blocked this effect of nerve stimulation. Brown (1967) carried out experiments in cats and dogs that were similar to those of Flacke and Gillis, except he also recorded postganglionic action potentials as a measure of response. These experiments of Flacke and Gillis, and of Brown, demonstrated conclusively that muscarinic (atropine sensitive) transmission can occur within the stellate ganglion of cats or dogs in the presence of non-depolarizing ganglionic blocking agents.

The purpose of this investigation was to analyze impulse transmission within the SCG in a manner analagous to the methods previously discussed, particularly with respect to techniques used to study impulse

transmission and activation of muscarinic pathways. In addition to studying muscarinic mechanisms affecting contraction of the NM, we also studied sympathetic activation of the parasympathectomized pupil. This investigation was unique in that responses of both effector organs were recorded simultaneously during 1) unilateral stimulation of the preganglionic (SCG) nerve, and 2) administration of specific ganglionic agonists and antagonists.

METHODS

General Procedure

Cats of either sex were anesthetized with alpha-chloralose (50 to 70 mg/kg i.p.). The animals were placed in a stereotaxic holder in order to facilitate surgical and experimental procedures. A femoral artery and vein, as well as the trachea, were cannulated. Systemic arterial blood pressure was recorded from the femoral artery with a Statham P-23 transducer. In some experiments it was necessary to administer gallamine triethiodide (2.5 to 5.0 mg/kg i.v.) in order to reduce artifacts due to activation of extraocular muscles. Respiration was then maintained with the aid of a constant volume ventilator (Harvard Apparatus).

Contractions of the NM were recorded with a Grass FT.03 force displacement transducer. An initial tension of 10 grams was placed on the muscle (Westfall et al., 1969). Changes in pupillary diameter were recorded with the aid of an electronic pupillometer modified from the original design of Dennison and Schaeppi (1967). Responses were monitored in cats that had undergone parasympathectomy, as well as those in which the 3rd C.N. was left intact. In the parasympathectomized preparations the postganglionic short ciliary nerves (medial and lateral) lying within the ocular orbit were sectioned. A lateral approach similar to that outlined by Nisida and Okada (1960) was followed. In these preparations, the pupil was constricted by topical application of one or two drops of a

1 per cent solution of physostigmine (Ury and Gellhorn, 1939). The cervical sympathetic nerve trunk was sectioned preganglionically in all preparations. All measurements were recorded on a Grass polygraph (Model 7B).

Electrical Stimulation

Bipolar platinum electrodes were placed beneath the cervical sympathetic preganglionic nerve after it had been separated from adjacent nerve trunks and the nerve was covered with warm mineral oil. The stimulus was derived from a Grass stimulator (Model S8) coupled to a Grass isolation unit. The stimulus strength was supramaximal (8-12 V) for 8 seconds. The pulse width was 1 msec. duration and the frequency was varied between 0.5 and 64 Hz.

Pharmacological Procedures

NM and pupillary responses were recorded simultaneously following i.a. administration of DMPP and McN-A-343, alone and in the presence of their specific antagonists. Responses were monitored in cats in which the 3rd C.N. was left intact, as well as in cats which had been parasympathectomized. Changes in NM tension and pupillary diameter were elicited with relatively small doses of DMPP and McN-A-343 administered i.a. close to the ganglion. Drugs were injected into the lingual artery in small volumes (0.10 to 0.20 ml). They were then directed to the SCG by occluding the external carotid just above its junction with the lingual artery, or to the NM and systemic circulation by not occluding the external carotid artery. The procedure was similar to that outlined by

Trendelenburg (1956). Figure 1 illustrates the efficacy of such an injection procedure. A 2 μg dose of epinephrine delivered to the ganglion had no effect on either the NM or the pupil, whereas the same dose delivered to the membrane elicited responses in both organs.

Drugs Used

All drugs were dissolved in normal saline and the doses are expressed in terms of their salts. Drugs used and their source were as follows: alpha-chloralose (Nutritional Biochemicals Corp.); 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, Aldrich Chemical Co., Inc.); 4-(m-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343, McNeil Laboratories, Inc.); hexamethonium chloride (C-6, Nutritional Biochemicals Corp.); atropine sulfate (Nutritional Biochemicals Corp.); physostigmine salicylate (Sigma Chemical Co.); gallamine triethiodide (American Cyanamid Co.); and epinephrine hydrochloride (Parke, Davis and Co.).

RESULTS

Response of the NM and Pupil to Electrical Stimulation

Contractions of the right and left NM were recorded simultaneously during electrical stimulation of the preganglionic sympathetic nerve on one side and the postganglionic sympathetic nerve on the other. The response of the two membranes to variable frequencies of stimulation during one experiment is shown in Figure 2. Contractions of both membranes were immediate and complete relaxation occurred rapidly after cessation of stimulation. C-6, infused at a rate of 0.60 mg/kg per min., effectively blocked NM contractions elicited by preganglionic stimulation at low frequencies, but only reduced the responses to higher frequencies of stimulation. Subsequent administration of atropine, 500 µg/kg i.v., completely blocked the response at all frequencies of stimulation. As expected, neither antagonist had any effect on responses elicited by postganglionic stimulation. Similar results were obtained in 9 other preparations.

C-6 administered as an i.v. infusion at a constant rate no greater than 1.03 mg/kg per min. always effected a reduction in the response to preganglionic stimulation. The magnitude of the reduction ranged from 100 per cent at lower frequencies to between 87 and 59 per cent at the highest frequencies of stimulation. Subsequent administration of atropine produced further reductions in all preparations. Little or no change in

the frequency-response curves were seen when atropine alone was administered in doses ranging between 0.5 and 2.0 mg/kg i.v.

In another series of experiments, the preganglionic sympathetic nerve trunk was stimulated unilaterally at variable frequencies while simultaneously recording contractions of the NM and dilation of the pupil on the same side. A typical response of the two effector organs to preganglionic stimulation is shown in Figure 3. In this preparation the 3rd C.N. was crushed postganglionically within the orbit and the pupil constricted with physostigmine. Note the characteristic difference in the threshold of activation between the two effector organs. In this preparation the NM responded to a stimulus of less than 1 Hz, whereas the pupil was unresponsive until the frequency was increased to 4 Hz. On the other hand, maximal response was attained at 32 Hz for the pupil, but not until 48 Hz for the membrane.

Figure 4 is a record obtained in another cat prepared in a similar manner. The variability in the threshold of activation between the NM and pupil, as shown in the preceeding figure, is again apparent. C-6, infused at a rate of 0.90 mg/kg per min., resulted in a complete blockade of pupillary responses, but only a reduction of the NM responses. When atropine was subsequently administered in a single dose of 2 μg, i.a., the NM responses were blocked completely.

Figure 5 summarizes the results of six experiments carried out as previously discussed. Responses at a given frequency of stimulation were plotted in terms of per cent of maximal response. The frequency-response composite curves show the mean values plus and minus standard errors. The difference in the threshold of activation for the NM and

pupil are again clearly visible. The intensity of stimulation necessary to effect a response equal to 50 per cent of maximal for the NM was 3.4 Hz, whereas it was 7.0 Hz for the pupil. Differences in the completeness of C-6 blockade at variable frequencies of stimulation are also clearly visible. At a stimulation frequency of 24 Hz the response of the pupil is only 6 per cent of maximal, whereas for the NM it is 24 per cent of maximal. The effectiveness of a subsequent dose of atropine in abolishing this residual transmission is evident.

Response of the NM and Pupil to Pharmacological Activation of Ganglionic Receptors

Figure 6 illustrates the response of the two effector organs to McN-A-343 and DMPP administered i.a. to the ganglion. As before, the 3rd C.N. had been crushed postganglionically and the pupil constricted by topical application of physostigmine. The effect of a 10 μg dose of DMPP was similar to that elicited by supramaximal stimulation of the preganglionic nerve. Contraction of the NM and dilation of the pupil was brisk and transient. The magnitude of the response is equally as great as that elicited by electrical stimulation. In contrast, the response elicited by a 30 μg dose of McN-A-343, as shown by contraction of the NM, is relatively slow in onset and long in duration. The magnitude is approximately one-fourth that elicited by electrical stimulation. Note the lack of response of the pupil in this example. This selective activation of the NM with respect to the pupil was observed in both types of experimental preparations; ones in which the 3rd C.N. was crushed postganglionically within the orbit, as well as in animals where the nerve was left intact.

Mean responses of the NM and pupil to single i.a. injections of McN-A-343 and DMPP in parasympathectomized cats are shown in Figure 7. The mean response of the NM following i.a. administration of 30 to 40 μg of McN-A-343 in 6 cats was 25.9 ± 3.1 per cent, compared with 6.2 ± 2.8 per cent of maximal for the pupil. The mean response of the NM and pupil to a 10 μg dose of DMPP administered i.a. in 8 cats was near maximal in both instances, being 95.4 ± 2.6 and 94.7 ± 3.1 per cent, respectively.

DISCUSSION

Prior investigators have presented considerable evidence demonstrating that muscarinic receptors within sympathetic autonomic ganglia can be activated by i.a. administration of specific agonists such as muscarine and McN-A-343. Furthermore, it has been shown that impulse transmission in sympathetic ganglia may occur via muscarinic as well as the classical nicotinic pathways. In our first series of experiments we utilized the techniques advanced by Flacke and Gillis (1968) in studying the stellate ganglion. Our results in regard to impulse transmission via muscarinic pathways in the SCG are qualitatively similar to their results in that C-6 did not completely block NM contractions evoked by stimulation of the preganglionic nerve at high frequencies. Quantitatively, our results are not similar to those of Flacke and Gillis in that we were unable to obtain a near maximal response at high frequencies of stimulation in the presence of C-6 blockade. In studies regarding transmission in the SCG during the late non-depolarizing phase of nicotinic blockade, Trendelenburg (1966) was able to obtain a NM response of only 32 per cent of maximal during supramaximal preganglionic stimulation. Our results are also supported by the observations of Chinn and Weber (1974) in that the largest NM response they were able to elicit was only 30 per cent of maximal during C-6 blockade. Thus it seems to be well established that there are quantitative differences in the degree of muscarinic transmission possible in sympathetic ganglia.

Impulse transmission via muscarinic receptors has been demonstrated by other investigators using a more physiological approach. Hilton and Steinberg (1966) studied the effects of several ganglionic blocking agents on the pressor response elicited by elevation of the intracranial pressure. They observed that chlorisondamine, administered alone, would not totally block the response and that a subsequent dose of 1.0 mg/kg of atropine was necessary for complete blockade. They concluded that non-depolarizing nicotinic ganglionic blocking drugs do not totally block all physiologic transmission and that an additional atropine sensitive pathway exists.

A unique part of our investigation was the simultaneous recording of NM and pupillary responses during unilateral stimulation of the preganglionic sympathetic nerve at variable frequencies. It was observed that 1) the threshold of activation was lower for the NM than for the pupil, 2) the slope of the frequency-response curve for the pupil was steeper than for the NM, and 3) C-6 was more effective in blocking pupillary than NM responses. The question was raised of whether or not measurable differences exist within the SCG in regard to activation of specific receptors affecting the two effector organs. An investigation relevant to differences in threshold of activation for all organs innervated by postganglionic fibers leading from the SCG was carried out by Bishop and Heinbecker in 1932. Their results indicate that the thresholds for activation of the pupil and NM are not very different, but that a 2 to 8 fold difference exists when comparing either of these with the blood vessels or pilomotor muscles, which are also innervated by postganglionic fibers leading from the SCG. Although their data does not lend support to

our hypothesis of differences in the threshold of activation for the NM and pupil, it does serve to emphasize that differences may exist for thresholds of activation of sympathetic postganglionic effector organs.

The second observation, concerning differences in the steepness or slope of the two frequency-response curves, is more difficult to evaluate with our data. These curves were obtained during what was believed to be nicotinic transmission within the ganglion. It would be of interest to compare electrically evoked frequency-response curves with nicotine agonist (DMPP)-response curves. If both effectors were to respond to DMPP in a similar fashion, one might be able to rule out the possibility of physiological antagonism.

The third observation, relating to NM and pupillary responses elicited by stimulation of the preganglionic nerve in the presence of C-6 or nicotinic blockade (muscarinic transmission), correlates well with data we obtained by McN-A-343 induced activation of receptors within the SCG. In the nerve stimulation studies, the presence of a non-depolarizing nicotinic type blocker was utilized to demonstrate the occurrence of muscarinic transmission within the SCG. The method was indirect and as noted earlier the presence of only a muscarinic type blocker had no effect upon ganglionic transmission initiated by preganglionic stimulation.

The advent of relatively specific ganglionic agonists, including the muscarinic agonist McN-343, afforded one additional approach in analyzing muscarinic mechanisms of impulse transmission and receptor activation within autonomic ganglia. Jones (1963) compared dose-response curves of this agent with nicotine and noted in one experiment that

McN-A-343 (300 μg i.a. to the SCG) elicited a NM response equal to 60 per cent of the maximal response elicited by nicotine. Trendelenburg (1966) noted that McN-A-343 (3 μg i.a. to the SCG) elicited a NM response equal to 17.6 and 22.9 per cent of that obtained by supramaximal preganglionic nerve stimulation and to administration of DMPP (3 μg i.a. to the SCG), respectively. Our data indicates that the maximal NM response to McN-A-343 is in the range of 25 per cent of maximal. This correlates well with values we obtained during preganglionic stimulation studies. Flacke and Fleisch (1970) found a similar correlation between the maximal response obtained following the administration of a large dose of McN-A-343 i.a. close to the stellate ganglion and that obtained by supramaximal stimulation of the preganglionic (stellate) nerve trunk in the presence of non-depolarizing blockade. In both of their procedures a near maximal response in heart rate was obtained.

The selective activation of the NM with respect to the pupil following the i.a. administration of McN-A-343 was surprising. The possibility exists that physiological (preparation with intact 3rd C.N.) or pharmacological (preparation with physostigmine pupillary constriction) antagonism is the cause of the higher threshold of activation for the pupil. On the other hand, it is possible that one may selectively activate one postganglionic effector organ without affecting the other. Koss and Wang (1972) have shown that stimulation of specific areas in the brainstem of the cat will elicit contraction of the NM without a concomitant dilation of the pupils.

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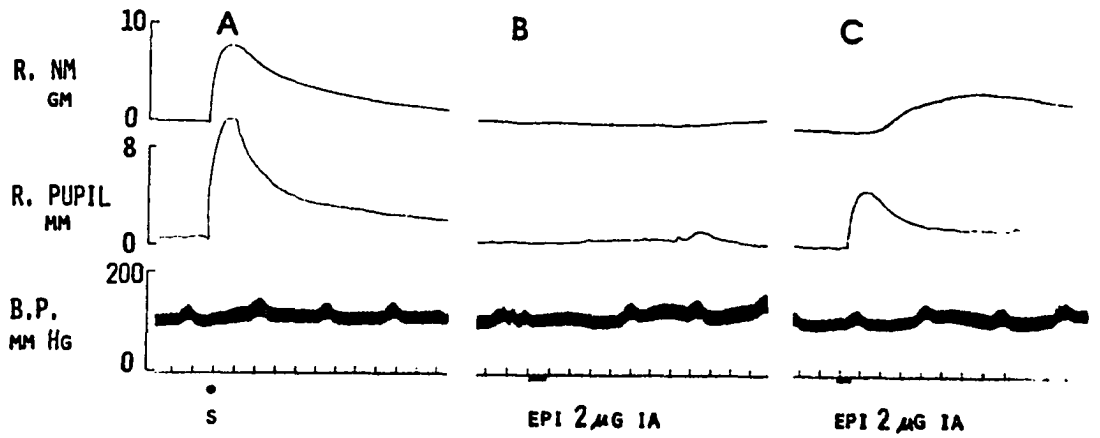

Figure 1. Response of the nictitating membrane and parasympathectomized pupil to electrical stimulation and to epinephrine administered via the lingual artery. A: Response elicited by supramaximal electrical stimulation of the sympathetic preganglionic nerve (S). B: Response elicited by 2 µg of epinephrine delivered to the superior cervical ganglion by occluding the external carotid just above its bifurcation with the lingual artery. C: Response elicited by 2 µg of epinephrine delivered to the membrane and pupil by not occluding the external carotid artery. Time mark each 5 seconds.

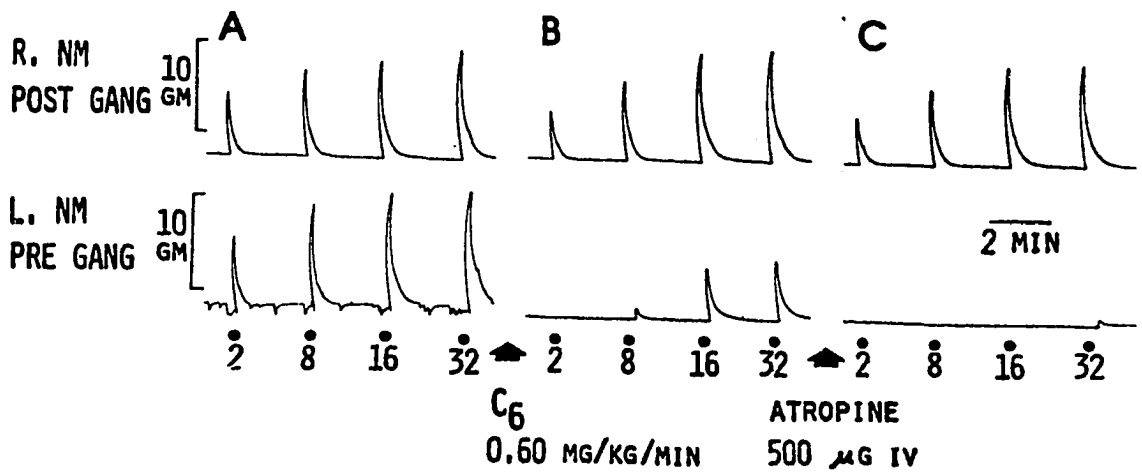

Figure 2. Response of the right and left nictitating membrane to electrical stimulation of the postganglionic (upper row) and preganglionic (lower row) sympathetic nerve at variable frequencies (2 to 32 Hz). A: Control responses. B: Responses during infusion of C-6, 0.60 mg/kg per min. C: Responses following subsequent administration of atropine, 500 µg/kg i.v.

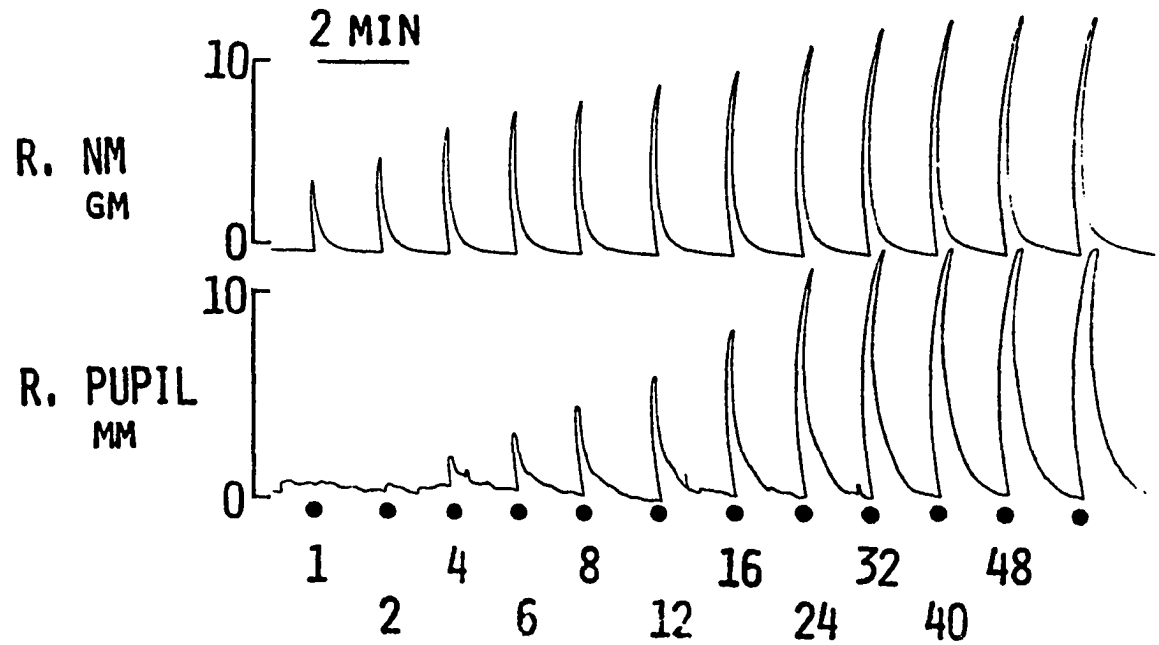

Figure 3. Response of the right nictitating membrane and right pupil to stimulation of the preganglionic sympathetic nerve at variable frequencies (1 to 64 Hz).

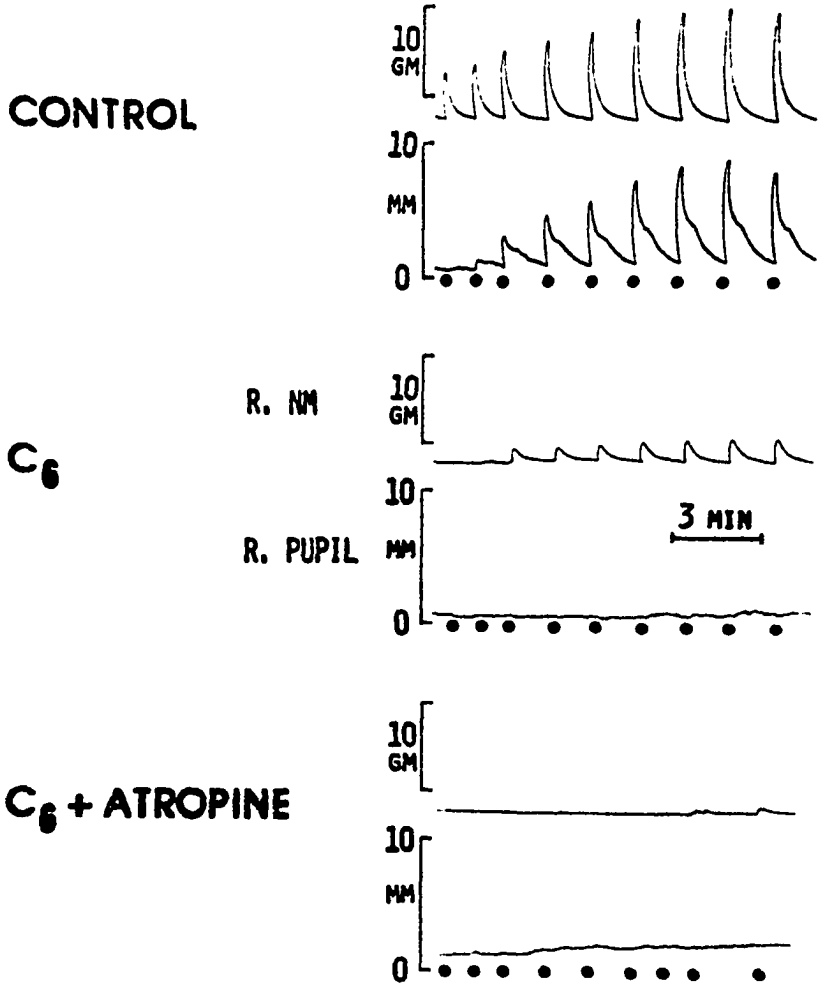

Figure 4. Effects of C-6 and C-6 plus atropine on nictitating membrane contraction (upper rows) and pupillary dilation (lower rows) elicited by supramaximal stimulation of the preganglionic sympathetic nerve at variable frequencies (1.0 to 32 Hz). Top two rows are control responses. Middle two rows are responses during i.v. infusion of C-6 (0.90 mg/kg) per min. Bottom two rows are responses in the presence of both C-6 and atropine (2 μg i.a., to the ganglion).

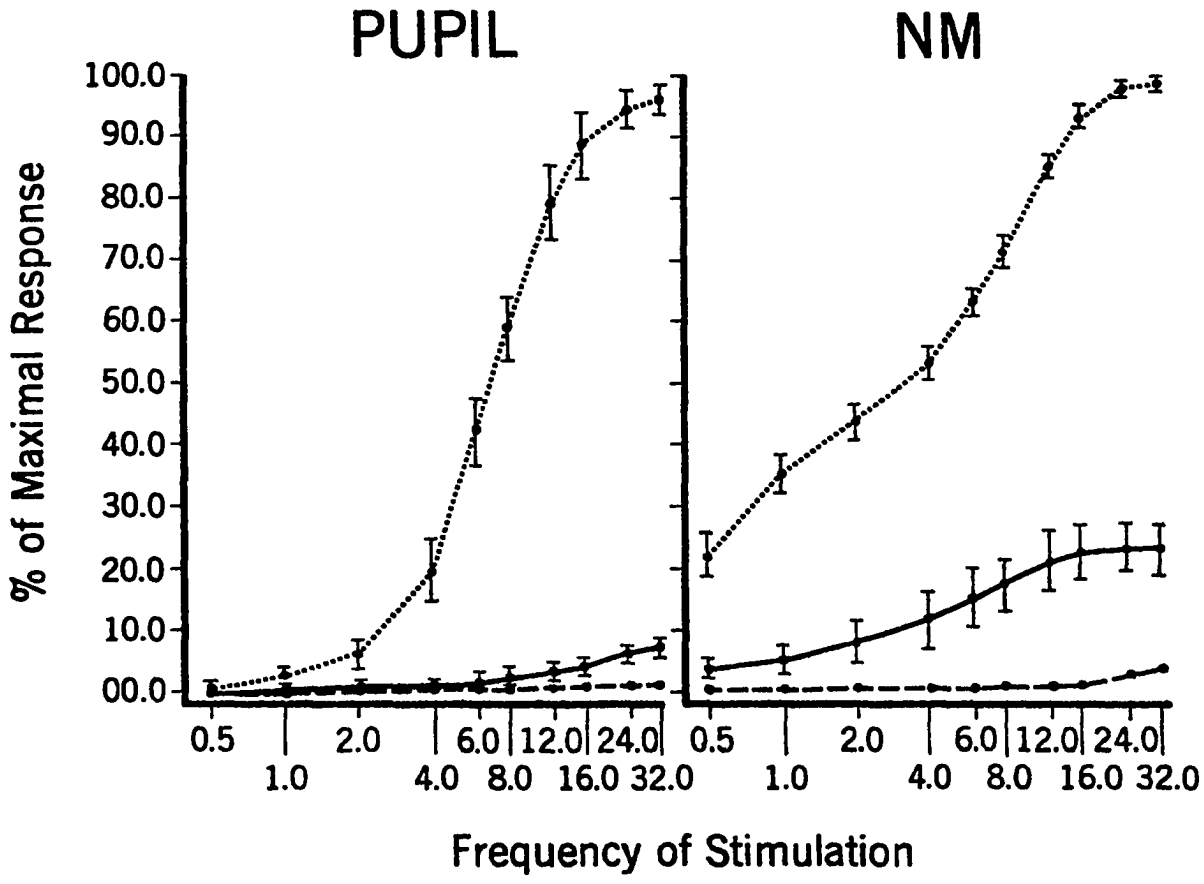

Figure 5. Frequency-response curves representing responses of nictitating membrane and pupil to variable frequency of stimulation of preganglionic sympathetic nerve trunk. Curves plotted from mean values ± S.E. of six experiments. Upper curves (.....) represent control responses. Middle curves (·————·) represent responses after an infusion of C-6. Bottom curves (·-------·) represent responses after administration of atropine (2 to 4 μg i.a.) during the infusion of C-6.

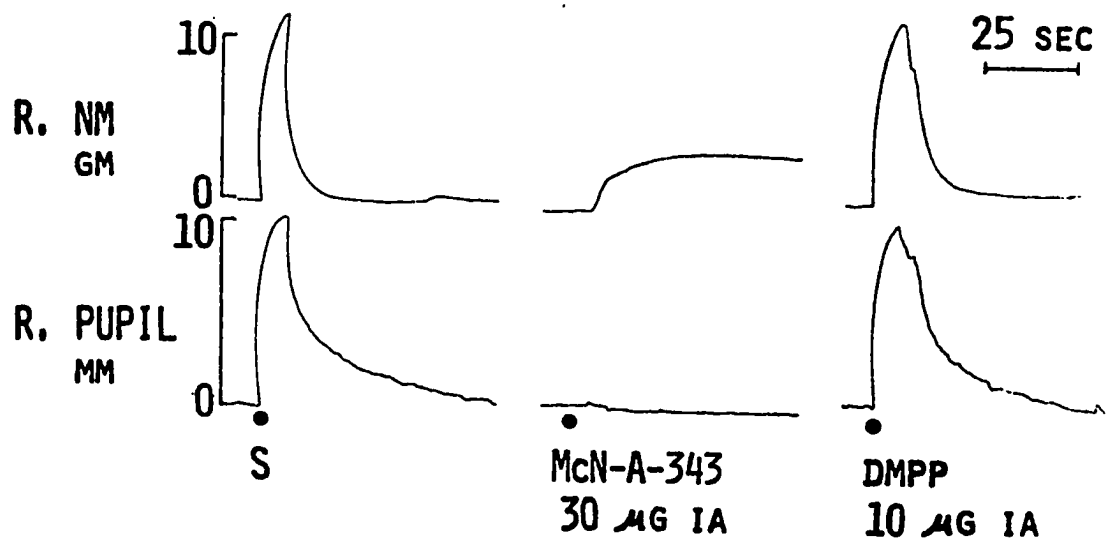

Figure 6. Response of the right nictitating membrane (upper row) and right pupil (lower row) to stimulation of the preganglionic nerve (S); to McN-A-343 (30 µg i.a.); and to DMPP (10 µg i.a.).

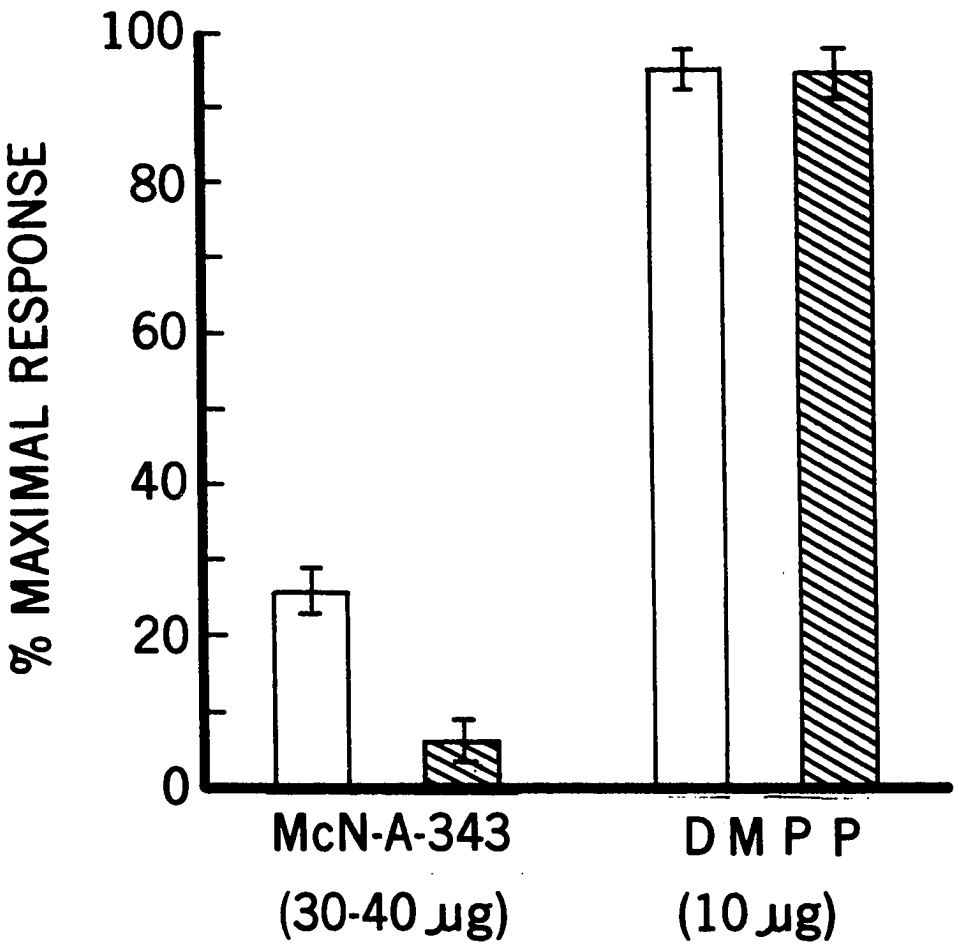

Figure 7. Response of the nictitating membrane (open bars) and pupil (hatched bars) to McN-A-343 and DMPP administered i.a. to the ganglion. Vertical lines are ± S.E. N=6 for McN-A-343 and 8 for DMPP.

CHAPTER III

MUSCARINIC MECHANISMS IN THE CILIARY GANGLION OF THE CAT

ABSTRACT

An investigation was carried out to determine whether or not muscarinic mechanisms are involved in impulse transmission within the ciliary ganglion. Pupillary constriction, evoked by variable frequency of stimulation of the preganglionic oculomotor nerve was blocked completely by the administration of C-6 (2 to 10 mg/kg). Subsequent administration of physostigmine salicylate (0.25 to 0.50 mg/kg) failed to reverse this C-6 blockade. Pupillary constriction was also elicited by intra-arterial and intravenous administration of the nicotinic agonist DMPP and the muscarinic agonist McN-A-343. Experiments were designed to ascertain whether McN-A-343 was acting on receptors within the ciliary ganglion or directly on the iris. Evidence strongly supports the concept of a direct action of McN-A-343 on the iris. Further evidence in support of this conclusion was obtained by recording changes in postganglionic potentials from the short ciliary nerves following the administration of DMPP and McN-A-343. Activation of the ciliary ganglion was observed following the administration of DMPP but this ganglion was unresponsive to large doses of McN-A-343. These results indicate that unlike sympathetic ganglia, transmission does not occur by means of a muscarinic mechanism in the ciliary ganglion.

INTRODUCTION

The existence of cholinergic mechanisms of the muscarinic type in sympathetic autonomic ganglia has been demonstrated by several groups of investigators. Ambache et al. (1956) were among the first to demonstrate that muscarine, administered intra-arterially (i.a.) close to the superior cervical ganglion (SCG), would elicit a contraction of the cat's nictitating membrane (NM). Additional evidence was presented by Roszkowski (1961) when he introduced the compound McN-A-343 and demonstrated that it too would activate muscarinic receptors within the SCG of the cat. Flacke and Fleisch (1970) studied the inter-relationships between the agonists DMPP and McN-A-343 and the antagonists C-6 and atropine in regard to their action on receptors within the stellate ganglion of the dog. They concluded that sympathetic autonomic ganglia possess both nicotinic and muscarinic excitatory mechanisms which can be activated and blocked selectively.

It also has been shown that impulse transmission, generated by stimulation of the preganglionic nerve trunk, can occur within sympathetic ganglia via muscarinic pathways. Flacke and Gillis (1968) obtained frequency-response curves in dogs by stimulating the preganglionic nerve to the stellate ganglion while recording changes in heart rate. They demonstrated that in the presence of non-depolarizing nicotinic blocking agents, frequency-response curves were shifted to the right with near

maximal responses still obtainable. Similar results were observed in cats (Brown, 1967). In contrast, studies on impulse transmission via muscarinic pathways within the SCG of cats using similar experimental procedures have shown that although impulse transmission can occur, it is of a lesser degree (Chin and Weber, 1974; Chapter II).

In parasympathetic ganglia, little is known in regard to the presence of muscarinic receptors or the possible transmission of impulses via these receptors. Saxena (1971) has shown that i.a. administration of McN-A-343 and AHR-602, another ganglionic muscarinic agonist introduced by Franko et al. (1963), will elicit dose dependent contractions of the urinary bladder in dogs. The stimulant action of the above drugs was blocked not only by a low dose of atropine (5 μg/kg), but also by depolarizing ganglionic blockade induced by nicotine, TMA, or DMPP. Taira et al. (1971) have shown that McN-A-343 induced bladder contractions in dogs can be blocked by small doses of atropine or by tetrodotoxin but not by C-6 or TEA. Other investigators have failed to obtain evidence supporting the concept of muscarinic receptors in parasympathetic ganglia. Vanov (1965) observed no change in urinary bladder tension following i.v. administration of 10 μg of McN-A-343 to rats. Holman et al. (1971) noted that iontophoretic application of McN-A-343 to guinea pig pelvic ganglia failed to evoke spikes in cells which responded to acetylcholine and nicotine. Goldberg and DaCosta (1960) reported that bradycardia in dogs elicited by stimulation of the efferent vagus was blocked completely by a 2 mg/kg dose of C-6.

This investigation was undertaken in order to determine by more direct means whether or not muscarinic activation and impulse transmission

via muscarinic pathways occurs in parasympathetic ganglia. The problem of ascertaining the site of action of muscarinic agonists and antagonists is complicated by the fact that acetylcholine serves as the neurotransmitter at the postganglionic neuroeffector junction, as well as at the ganglionic junction. There are only two sites within the parasympathetic system where postganglionic nerve fibers are of sufficient length to permit direct recording of nerve activity: 1) the fibers arising from the ciliary ganglion which innervate the sphincter muscle of the pupil, and 2) the fibers arising from the otic ganglion which innervate the parotid gland. Recording of changes in postganglionic potentials from either of these sites following the administration of muscarinic ganglionic agonists would give direct evidence for or against the concept of muscarinic receptors being present in parasympathetic ganglia. The ciliary ganglion of the cat was chosen for several reasons, including 1) it is accessible within the orbit, 2) it has been shown that no sympathetic fibers run through this ganglion (Christensen, 1936), and 3) several investigators (Whitteridge, 1937; Nisida and Okado, 1960) have recorded from these postganglionic fibers and have provided some of the basic physiological groundwork. Finally, the direct and continuous monitoring of pupillary changes (with the aid of an electronic pupillometer) will enable measurements of both a qualitative and quantitative nature.

METHODS

Cats of either sex were anesthetized with alpha-chloralose (50 to 70 mg/kg i.p.). The animals were placed in a stereotaxic holder in order to facilitate surgical and experimental procedures. A femoral artery and vein, as well as the trachea, were cannulated. Systemic arterial blood pressure was recorded from the femoral artery with a Statham P-23 transducer. In some experiments, contraction of the NM was recorded with the aid of a FT.03 Grass force displacement transducer. An initial tension of 10 grams was placed on the muscle (Westfall et al., 1969). Changes in pupillary diameter were recorded with the aid of an electronic pupillometer modified from the original design of Dennison and Schaeppi (1967). In some experiments, gallamine triethiodide (2.5 to 5.0 mg/kg i.v.) was administered in order to reduce artifacts arising from movement of the extraocular muscles. In these cases respiration was maintained with the aid of a constant volume ventilator (Harvard Apparatus). All measurements were recorded on a Grass polygraph (Model 7B).

Surgical Procedure

Access to the ciliary ganglion (Fig. 1), as well as to its pre- and postganglionic nerve trunks, was achieved via a lateral approach. Beginning at the lateral edge of the eye, a V shaped incision was made

which extended in a posterior direction about 6 cm. Cauterization was used to minimize bleeding. The underlying skeletal musculature, including the deep temporalis muscle which was detached at its rostral end, was ligated in several places and retracted in a posterior direction. The zygomatic arch and part of the mandibular process was removed. The orbital capsule was incised, and several of the extraocular muscles were ligated and retracted in order to expose the ciliary ganglion, as well as its pre- and postganglionic nerve trunks. Caution was exerted during the latter part of the procedure in order not to disrupt or damage the blood supply to the ganglia. An acceptable experimental preparation was obtained when the pupil remained constricted following completion of the surgical procedure and opened widely after crushing the preganglionic nerve trunk within the orbit or cranium. In the latter instance, a hole was first made in the skull with a trephine, a small slit made in the meninges, and then a long, flat probe with a blunt end was forced downward to the base of the cranium in order to crush the oculomotor nerve. Even though the above two criteria could be fulfilled, the circulatory system could still be damaged to such an extent that it prevented adequate concentrations of drugs from reaching the ciliary ganglion.

Electrical Stimulation

In some experiments bipolar platinum electrodes were placed beneath the oculomotor nerve within the orbit distal to a point where the nerve had been crushed. In others, the oculomotor nerve fibers were stimulated within the cranium by means of a coaxial electrode mounted on a electrode carrier, and positioned with the aid of a David Kopf stereo-

taxic instrument. In these cases the pupil was dilated by topical application of a few drops of a 5 per cent solution of ephedrine HCl. The preganglionic sympathetic nerve trunk leading to the SCG was always crushed in the intracranial stimulation experiments.

The stimulus was derived from a Grass stimulator (Model S8) coupled to a Grass isolation unit during intraorbital stimulation. A Grass stimulator (Model S48) and a constant current unit (Model PSIU6B) were utilized during intracranial stimulation. Stimulation parameters varied, depending on the sensitivity of the preparation.

Pharmacological Procedures

Pupillary responses to variable doses of DMPP and McN-A-343, administered i.a. or i.v., were monitored under several experimental conditions. Initially, attempts were made to elicit pupillary constriction in cats which had been given C-6. In another series, pupillary responses were recorded unilaterally in cats in which the oculomotor nerve had been cut preganglionically. In a third series, pupillary changes were monitored bilaterally following section of the oculomotor nerve preganglionically on one side and postganglionically on the other.

Direct effects of McN-A-343 were compared to those of acetylcholine on porcine irides in four in vitro assays. An initial 200 mg of tension was placed on the isolated muscle and agonist induced contractions were recorded with the aid of a force transducer (Myograph, Type A, E. & M. Instruments). The iris was superfused with warm (35°C), oxygenated Tyrodes solution containing known concentrations of agonists.

Recording Experiments

Postganglionic potentials were recorded utilizing bipolar platinum electrodes placed beneath one of the short ciliary (postganglionic) nerves. Drying of the nerve was prevented by covering it with cotton and bathing it with warm mineral oil. Electrical activity was amplified by a Tetronix differential amplifier (Model 26A2) which was coupled to a 1) Tetronix dual beam storage oscilloscope (Model D13), 2) Grass audio unit (Model AM8), 3) Grass integrator (Model 7510B), and 4) a Tandberg frequency-modulated tape recorder. Pictures were made of oscillographic tracings with the aid of a Polaroid C-5 camera. Figure 2 illustrates the correlation between the record as seen on the oscilloscope and that recorded on the polygraph with the aid of a Grass integrator.

Two types of stimuli were used to evoke changes in postganglionic potentials. 1) Chemical activation of both nicotinic and muscarinic receptors within the ciliary ganglion was attempted by i.a. and i.v. administration of DMPP and McN-A-343, respectively. 2) Photic stimulation (Grass photostimulator, Model PS22) of the contralateral eye was utilized in some experiments in order to study the effects of C-6 and atropine on postganglionic potentials evoked in the short ciliary nerves by means of the consensual light reflex. In these latter experiments the short ciliary nerves were crushed on the side of photic stimulation in order to maintain a constantly dilated pupil.

Drugs Used

Drugs were dissolved in normal saline and the doses are expressed in terms of their salts. Drugs employed were: acetylcholine chloride

(Sigma Chemical Co.); alpha-chloralose (Nutritional Biochemicals Corp.); 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, Aldrich Chemical Co., Inc.); 4-(m-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride (McN-A-343, McNeil Laboratories, Inc.); hexamethonium chloride (C-6, Nutritional Biochemicals Corp.); ephedrine hydrochloride (Abbott Laboratories); atropine sulfate (Nutritional Biochemicals Corp.); physostigmine salicylate (Sigma Chemical Co.); gallamine triethiodide (American Cyanamide Co.).

RESULTS

Response of the Pupil to Electrical Stimulation of the Preganglionic Nerve

Frequency-response curves were obtained in 5 cats by stimulating the oculomotor nerve within the cranium, after having dilated the pupil with ephedrine hydrochloride. In all of these cases, C-6 completely blocked the constrictor response to nerve stimulation. Figure 3 is a typical example of a frequency-response curve obtained in this manner. It illustrates the efficacy of C-6 in completely abolishing responses elicited at all frequencies of stimulation. In these experiments, subsequent doses of physostigmine (0.25 to 0.50 mg i.v.) failed to reverse the C-6 blockade.

Frequency-response curves were also obtained in 7 cats by stimulating the 3rd C.N. preganglionically within the orbit, after having crushed the nerve distal to the point of electrode placement. Intravenous administration of C-6 in doses ranging from 5 to 10 mg/kg completely abolished responses elicited by all frequencies of stimulation in only 4 of these experiments. In the 3 remaining instances, there was evidence of circulatory insufficiency following the extensive surgical procedures in that C-6 did not abolish the responses elicited at any frequency of stimulation.

Pupillary Responses to Chemical Agonists

The response of the pupil to DMPP and McN-A-343, administered i.a. via the lingual artery, is shown in Figure 4. A 10 μg dose of DMPP elicited a prompt and transient constriction of the pupil. A 200 μg dose of McN-A-343 elicited a minimal pupillary constriction which was characteristically slow in onset and relatively long in duration. A 10 mg/kg dose of C-6 administered i.v. abolished the DMPP induced responses. Changes in blood pressure were much less than would have occurred following i.v. administration of these agonists.

The response of the pupil to McN-A-343, administered i.a. in variable doses in 6 other cats, was similar to that shown in Figure 4. Regardless of whether the 3rd C.N. was crushed preganglionically or whether C-6 was administered to induce pupillary dilation, the response to McN-A-343 was always minimal, i.e. 1 to 3 mm of constriction.

The results of 4 experiments designed to ascertain the site (s) of action of the McN-A-343 induced pupillary constriction are shown in Figure 5. In these cats the 3rd C.N. was sectioned preganglionically on one side and postganglionically on the other side. Possible sites of action for McN-A-343 in this preparation are 1) the ciliary ganglion and the iris on the side of preganglionic section and 2) only the iris on the side of postganglionic section. Responses of both pupils were recorded simultaneously following the i.v. administration of 500 μg/kg of McN-A-343. Curves, depicting mean responses plus or minus standard errors for both pupils during a 8 minute testing period are shown in Figure 5. The response was slow in onset and long in duration. No more than 3 mm of constriction was seen in either pupil. There was no significant

difference in the degree of pupillary constriction at any given point in time after the administration of McN-A-343.

Direct effects of McN-A-343 on isolated porcine irides were observed in 4 experiments. Concentrations of McN-A-343 ranging from 20 to 200 µg/ml effected graded increases in tension until a maximum of 200 to 250 mg. was reached. The efficacy of McN-A-343 was less than that of acetylcholine which elicited a maximal response of 500 mg. The dose-response curve for acetylcholine was much steeper than that of McN-A-343. The potency of McN-A-343 was approximately 1/150 that of acetylcholine.

Postganglionic Nerve Recording

McN-A-343 failed to elicit any measurable increase in postganglionic potentials while DMPP consistently evoked large increases in the firing rates recorded from the short ciliary nerves. Figure 6 illustrates a typical response obtained in 6 cats given large i.v. doses of DMPP (50 to 150 µg/kg) and McN-A-343 (100 to 400 µg/kg). In two cats, changes in postganglionic potentials were recorded from the lateral short ciliary nerve on one side, while contractions of the NM were recorded on the other side. Results of one such experiment are shown in Figure 7. Postganglionic potential increases, as well as the contraction of the NM elicited by DMPP, were completely blocked by C-6. No increase in postganglionic potentials was observed following the i.v. administration of McN-A-343, 400 µg/kg, even though the NM contracted in a typical manner, i.e. with a slow onset and long duration. As expected, C-6 did not diminish the contraction of the NM elicited by McN-A-343.

Experiments were carried out in 4 cats wherein changes in post-

ganglionic potentials were elicited by photic stimulation of the contralateral eye. Bi- and tri-phasic potentials recorded from the short ciliary nerves similar to those described by Sigg and Sigg (1973) were completely abolished in the presence of C-6 (5 mg/kg i.v.). As before, McN-A-343 remained without effect after ganglionic blockade with C-6.

DISCUSSION

The existence of muscarinic receptors, as well as transmission via muscarinic pathways, has been demonstrated in sympathetic ganglia by numerous investigators (Chapter II). Two basic techniques have been utilized in most studies relating to receptor activation of impulse transmission in autonomic ganglia. The first involves i.a. administration of relatively small doses of agonists and/or antagonists, "close to the ganglion" as described by Trendelenburg (1966). The second involves generation of impulse traffic within a given ganglion by stimulating the preganglionic nerve at variable frequencies while measuring postganglionic effector responses. (These responses can be measured by monitoring a physiological response or by directly observing the firing rate in postganglionic nerve fibers). The extent of impulse transmission via muscarinic pathways is determined by blocking as completely as possible the nicotinic receptors within the ganglion with non-depolarizing type blockers, and then repeating the sequence of preganglionic stimulation at variable frequencies. Frequency-response curves obtained under normal or control conditions can then be compared with transmission during complete nicotinic receptor blockade. The magnitude of the response at a given frequency of stimulation can be estimated in terms of per cent of maximal control responses.

These two basic techniques, in addition to several other

procedures, were used in attempts to resolve the question of whether or not impulse transmission via muscarinic pathways is possible in a parasympathetic ganglion of the cat. Our results are consistent with the hypothesis that muscarinic receptors are not present and transmission via muscarinic pathways does not occur within the ciliary ganglion of this species.

The effectiveness of C-6 in completely blocking pupillary responses elicited by preganglionic stimulation at variable frequencies of stimulation, as well as changes in postganglionic potentials elicited by photic stimulation of the contralateral eye, strongly supports our hypothesis. The ineffectiveness of physostigmine in reversing to any extent the C-6 blockade lends further support to this hypothesis. Several investigators have shown that physostigmine will enhance impulse transmission via muscarinic pathways in sympathetic ganglia (Fenner and Hilton, 1963; Gillis et al., 1968).

Impulse transmission within the ciliary ganglion has been previously studied by several investigators. Luco and Marconi (1949) observed that tetraethylammonium bromide "produced a reduction or abolition of the pupillary reaction" following preganglionic stimulation of the oculomotor nerve. In addition, Perry and Talesnick (1953) in their classical work delineating acetylcholine as the neurotransmitter in the ciliary ganglion, also observed that an i.v. injection of C-6 blocked all of the response of the pupil to preganglionic stimulation of the 3rd C.N. In these experiments the stimulus intensity was constant and of low frequency. In addition, pupillary function was completely blocked for only 2 or 3 minutes, recovering to about 45 per cent of control within 4

minutes following the injection of C-6. No frequency-response curves were obtained and no attempts were made to determine whether an alternate type of transmission (i.e. muscarinic) might be possible in this ganglion.

Flacke and Gillis (1968) and Brown (1967) demonstrated that frequency-response curves relating nerve stimulus frequency to heart rate increases were only shifted to the right in a parallel fashion in the presence of C-6. The same maximal increase in heart rate was achieved during transmission via either pathway (nicotinic or muscarinic), but at a higher frequency of stimulation during muscarinic transmission. In contrast, it has been demonstrated that frequency-response curves relating nerve stimulus frequency to contraction of the NM were not shifted to the right in a parallel fashion in the presence of C-6 blockade. The maximal increase in NM contraction during muscarinic transmission at highest frequencies of stimulation was only 25 to 30 per cent of the maximal achieved during nicotinic transmission (Chinn and Weber, 1974; Chapter II). Thus the extent of muscarinic transmission has been shown to vary in several sympathetic ganglia, but in no instance has it been shown to be totally absent.

Most of the evidence supporting the hypothesis of muscarinic transmission within parasympathetic ganglia has been obtained in studies on pelvic ganglia, although no attempts have been made to quantitate the extent of muscarinic transmission in this ganglion. Saxena (1971) reported that a dose dependent contraction of the dog's urinary bladder was elicited following i.a. administration of McN-A-343 (25-200 μg) and AHR-602 (0.1-1.0 mg). He also reported that a low dose of atropine (5 μg/kg),

as well as a depolarizing ganglionic blockade induced by nicotine, TMA, or DMPP, blocked the responses elicited by the two muscarinic agonists. Taira et al. (1971) has presented additional evidence in support of muscarinic receptors in pelvic ganglia of dogs. Intra-arterial injection of DMPP and McN-A-343 close to the ganglion elicited dose dependent contractions of the bladder. In most experiments the response to McN-A-343 was abolished by tetrodotoxin, indicating a neural mechanism of action. These results are in conflict with those of Vanov (1965) and Holman (1971) who were unable to activate muscarinic receptors in pelvic ganglia of rats and guinea pigs, respectively. This may however, reflect a species difference in the responsiveness to McN-A-343.

Impulse transmission within the parasympathetic ganglia of the heart has also been studied. Goldberg and DaCosta (1960) observed that bradycardia due to stimulation of the efferent vagus was blocked completely by a 2 mg/kg dose of C-6 in dogs. Chiba et al. (1971) reported an unusual observation, i.e., that McN-A-343 produced a dose related decrease in bradycardia induced by preganglionic stimulation of the vagus nerve, although McN-A-343 administered by itself induced a dose dependent bradycardia. Flacke and Gillis (1968) reported that stimulation of the vagal nerves with a supramaximal stimulus at increasing frequencies caused an increased bradycardia in the heart-lung preparation of the dog. Administration of doses of C-6 between 4 and 10 mg blocked this effect completely, but a subsequent injection of a small amount of physostigmine caused a fall in heart rate and a partial (25 to 50 per cent increase at higher frequencies of stimulation) return of the response to vagal stimulation. Small doses of atropine were effective in blocking the latter

responses.

As noted earlier, it is more difficult to study muscarinic receptor activation or transmission within parasympathetic ganglia compared to sympathetic ganglia because of the presence of cholinergic receptors at both synaptic junctions in the parasympathetic division. We were able to circumvent the problem by recording postganglionic potentials from the short ciliary nerves just distal to the ganglion. Although several other investigators have recorded ciliary postganglionic potentials (Whitteridge, 1937; Nisida and Okada, 1960), no one has attempted to measure ganglionic agonist induced changes. Our results show that DMPP, administered i.a. or i.v., consistently elicited increased firing in the postganglionic ciliary nerve whereas administration of variable doses of McN-A-343 failed to elicit any measurable change in postganglionic potentials. This is in contrast with similar studies concerning sympathetic ganglia. Sanghvi et al. (1963) and Murayama and Unna (1963) demonstrated that both DMPP and McN-A-343 elicited increases in postganglionic activity in the SCG and inferior mesenteric ganglia, respectively.

In the present study pupilloconstriction was consistently observed following the administration of McN-A-343 i.a. or i.v. Our data suggests that this pupilloconstriction is the result of a direct effect on the iris. The observation that no difference in pupillary response could be seen in preparations that had been parasympathectomized postganglionically on one side and preganglionically on the other indicates that the drug was acting beyond the ganglion. The observation that McN-A-343 elicited a contraction of the isolated porcine iris supports

such a hypothesis. In further support of this direct muscarinic action of McN-A-343, Roszkowski (1961) demonstrated that McN-A-343 was effective in stimulating the isolated guinea pig ileum as well as the frog rectus muscle (the relative potency was judged to be 1/200 and 1/40 of acetylcholine, respectively). Our assays on the porcine iris indicated the potency of McN-A-343 was approximately 1/150 that of acetylcholine, a value that compares favorably with those of Roszkowski.

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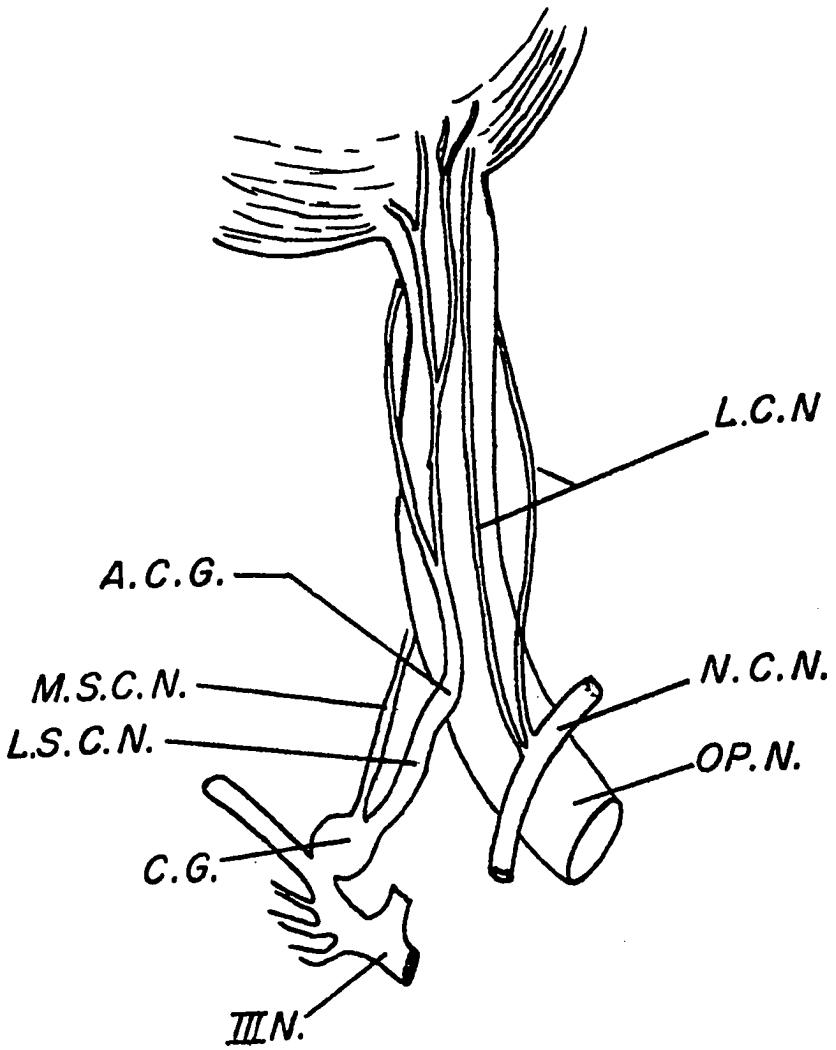

Figure 1. Diagram of ciliary ganglion (C.G.) and its relationship to other intraorbital nerves. Shown are the accessory ciliary ganglion (A.C.G.), medial short ciliary nerve (M.S.C.N.), lateral short ciliary nerve (L.S.C.N.), 3rd C.N. (III N.), optic nerve (OP.N), nasociliary branch of 5th C.N. (N.C.N.), and the long ciliary nerves (L.C.N.). (From Christensen, J. Anat., (London) 1936, 70: 225-232).

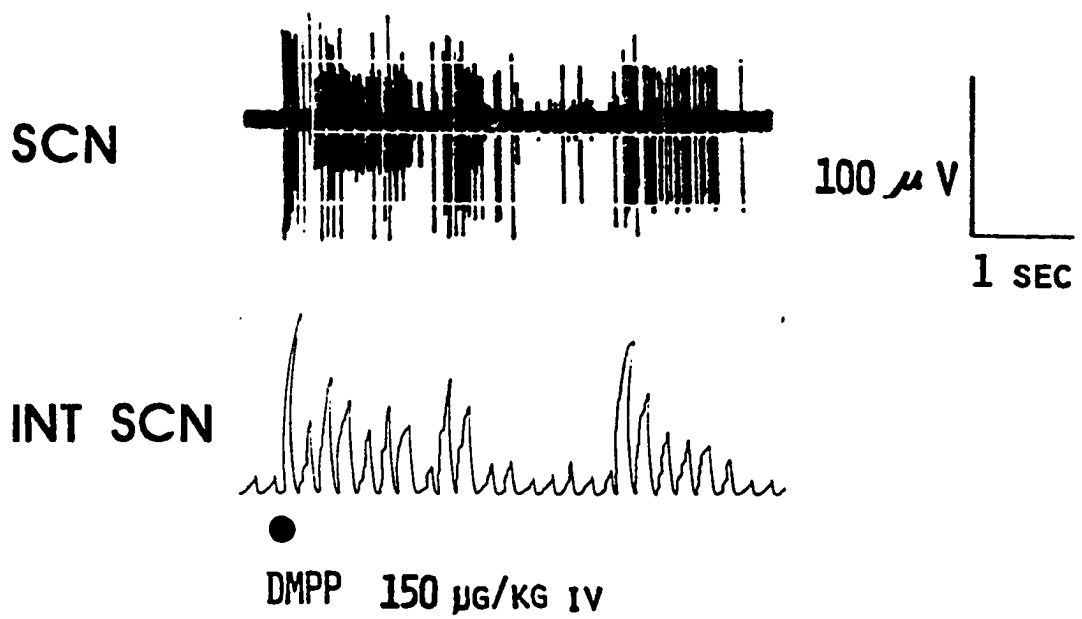

Figure 2. Potential changes recorded from lateral short ciliary nerve (SCN) within orbit of the cat following i.v. administration of 150 μg/kg of DMPP. Record illustrates correlation between response observed on oscilloscope (top) and that recorded on Grass polygraph with the aid of a Grass integrator (INT SCN).

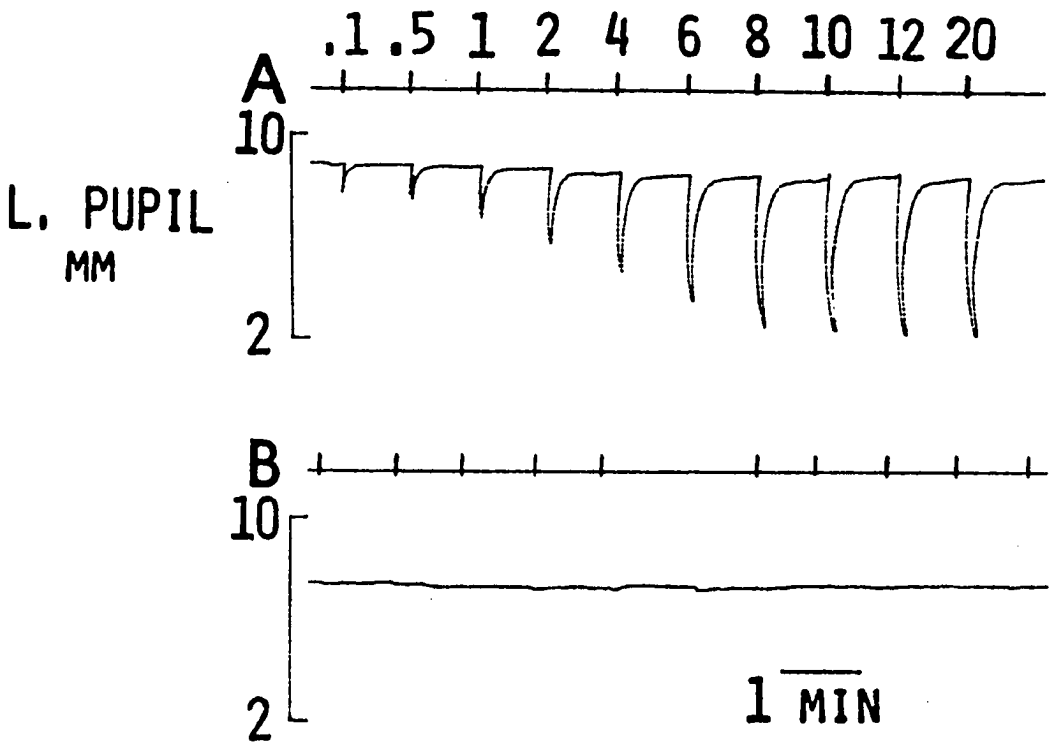

Figure 3. A: Response of the pupil to electrical stimulation of the oculomotor nerve within the cranium at variable frequencies, 0.1 to 20 Hz. B: Pupillary responses to same series of stimulation frequencies after 5 mg/kg of C-6 had been administered i.v.

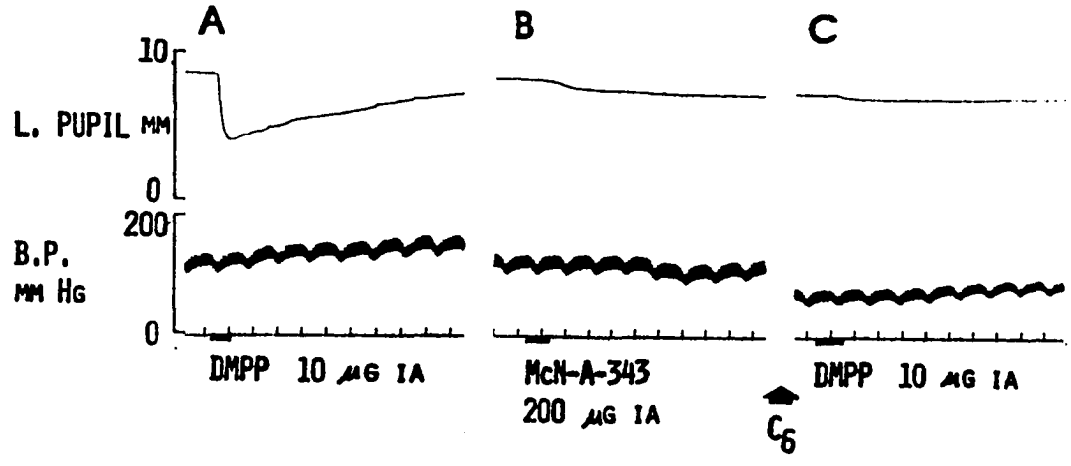

Figure 4. Effects of DMPP (10 µg i.a.) (A), and McN-A-343 (200 µg i.a.) (B), on pupillary diameter and blood pressure. Blocking action of C-6 (10 mg/kg i.v.) on DMPP induced pupillary constriction shown at (C). Time interval = 5 sec.

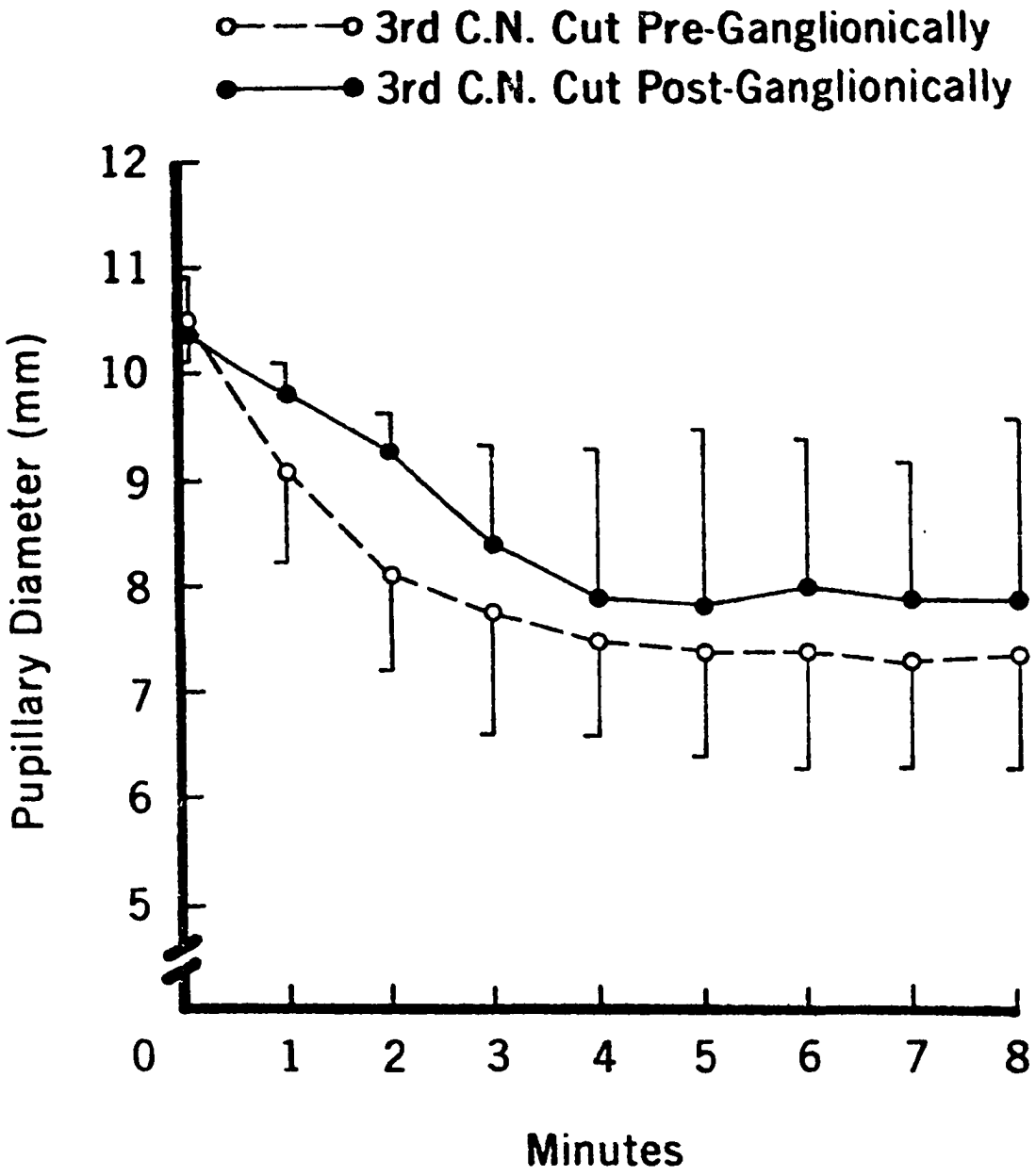

Figure 5. Effects of McN-A-343 (500 µg/kg i.v.) on pupillary diameter. Response of both pupils recorded simultaneously. The 3rd C.N. crushed within the orbit preganglionically on one side (o- - - -o) and postganglionically (·--------·) on the other. Points on curve represent means of four experiments. Vertical bars represent standard errors(S.E.).

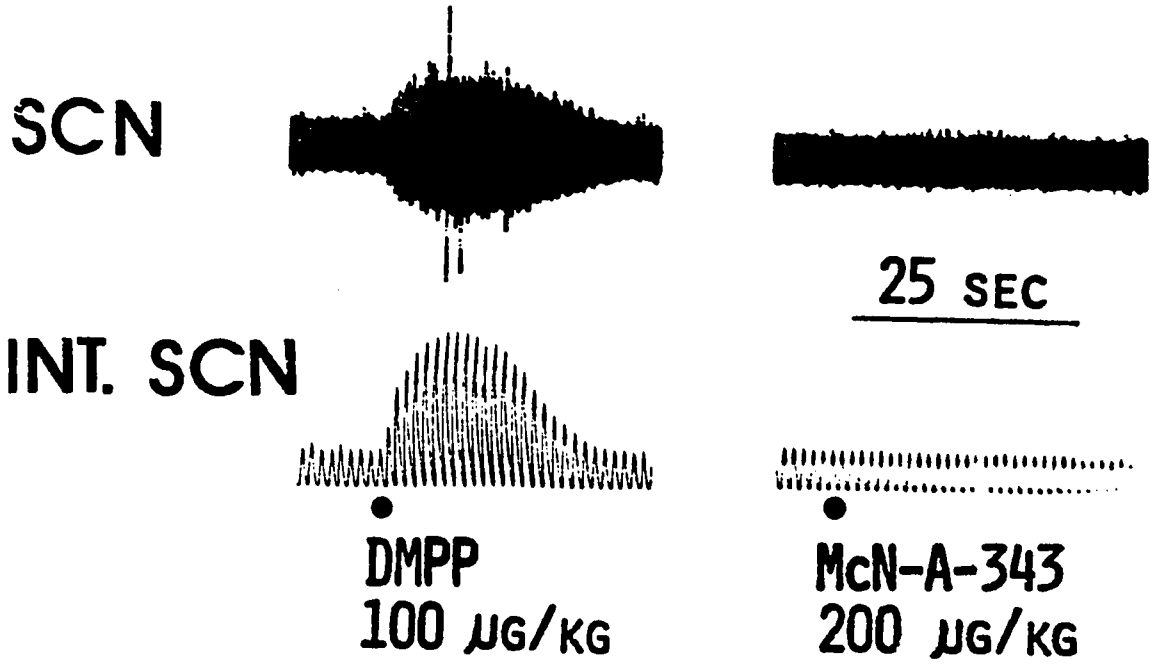

Figure 6. Effects of DMPP (100 µg/kg) and McN-A-343 (200 µg/kg) administered i.v., on postganglionic potentials recorded from the lateral short ciliary nerve. Top row: record as seen on oscilloscope. Bottom row: integrated potential as recorded on polygraph.

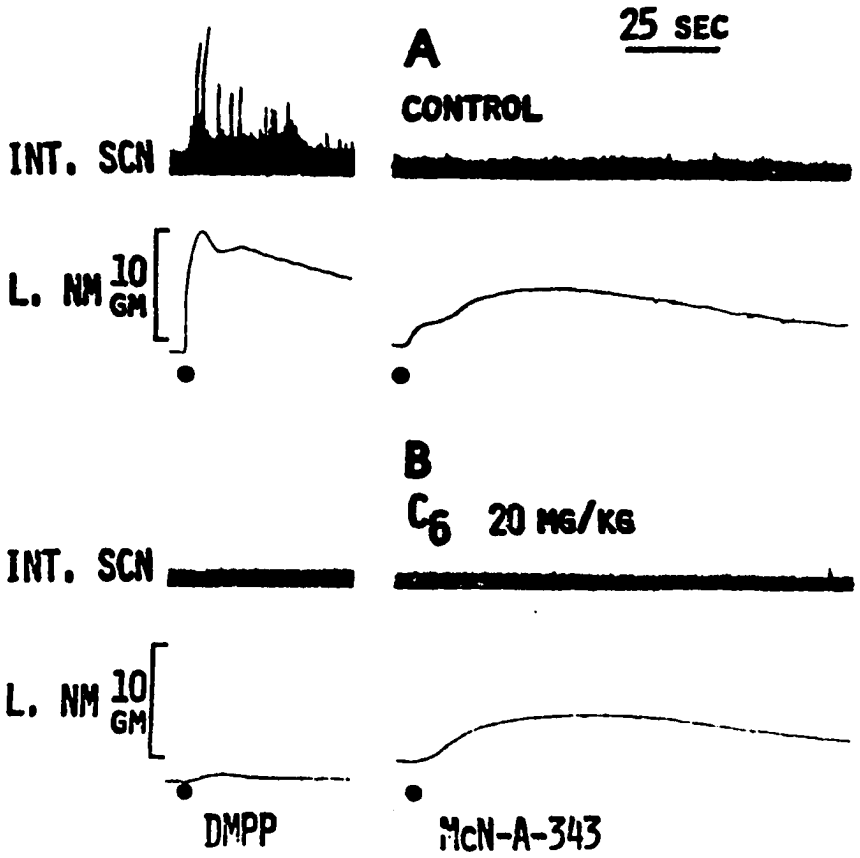

Figure 7. A: Effects of DMPP (100 µg/kg) and McN-A-343 (400 µg/kg) administered i.v., on postganglionic potentials recorded from lateral short ciliary nerve on right side and on NM recorded on left side. B: Responses recorded in identical manner after administration of C-6 (20 mg/kg) administered i.v.