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DISCRIMINATION BETWEEN NICOTINIC RECEPTORS IN VERTEBRATE GANGLIA AND SKELETAL MUSCLE BY ALPHA-BUNGAROTOXIN AND COBRA VENOMS

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SUMMARY

1. We have used snake neurotoxins, α-bungarotoxin and venoms from *Naja naja siamensis* and *Naja nivea*, to distinguish the nicotinic receptors of ganglia from those of skeletal neuromuscular junctions.

2. These neurotoxins failed to block responses of isolated guinea-pig longitudinal muscle with adherent myenteric plexus to the nicotinic agonists, nicotine or dimethylphenylpiperazinium, to acetylcholine (ACh), or to electrical field stimulation.

3. The toxins failed to affect responses of the isolated guinea-pig stomach to preganglionic stimulation by way of the vagus nerves or of the vas deferens to preganglionic stimulation via the hypogastric nerves.

4. Snake neurotoxins did not block non-adrenergic inhibitory responses of the rabbit small intestine to nicotine or electrical field stimulation.

5. Neurotoxins were ineffective blockers against nicotinic agonists in new-born rabbit or embryonic chick intestine.

6. Attempts to increase the penetration of the toxins into tissues with dimethylsulphoxide, exposure to hypertonic solutions, or to ethylenediaminetetracetic acid did not enable the toxins to act as nicotinic antagonists.

7. In contrast to diaphragmatic or oesophageal skeletal neuromuscular junctions no binding of rhodamine or tritium labelled toxins to structures in ganglia could be detected.

8. No potential permeability barriers were found by electron microscopy of the ganglia of the guinea-pig myenteric plexus.

9. The tracers, lanthanum ion and ruthenium red, readily penetrated into all regions of the myenteric plexus including synaptic gaps.

10. It is concluded that the failure of snake neurotoxins to act as

nicotinic antagonists or to bind to ganglia is not due to their inability to reach ganglionic nicotinic receptors. Therefore, it is likely that ganglionic nicotinic receptors are different from those of the skeletal neuromuscular junction.

INTRODUCTION

Receptors for acetylcholine (ACh) were classified by Dale (1937*a*) as nicotinic or muscarinic by analogy to the action of the alkaloids nicotine or muscarine (Dale, 1937*a*, *b*). Nicotine in low doses activat ines and higher doses blocks transmission both at autonomic ganglia and at the skeletal neuromuscular junction (Langley & Magnus, 1905; Bacq & Brown, 1937; Paton & Savini, 1968). The receptors mediating these responses to nicotine and to ACh are classically 'nicotinic'. However, differences between the nicotinic effects of drugs at ganglia and at the skeletal neuromuscular junction exist and have been appreciated for a long time (Koelle, 1975). For example, dimethylphenylpiperazinium (DMPP) and phenyltrimethylammonium (PTMA) are particularly selective agonists, and tetraethylammonium and hexamethonium antagonists, at ganglionic receptors. Decamethonium, in contrast, is a selective depolarizing antagonist at the skeletal neuromuscular junction but only a weak competitive antagonist at ganglia (Paton & Perry, 1953). Even nicotine differs in the details of its action at the two sites. At the skeletal neuromuscular junction the action of nicotine is predominantly stimulant and the drug seems to lack the intense and prolonged phase of self-antagonizing block characteristic of its action on ganglia (Paton & Savini, 1968). These differences between the action of drugs at nicotinic receptors in the two locations could be explained if the receptors are not, as their classification implies, the same.

The toxin obtained from *Bungarus multicinctus*, α-bungarotoxin (α-BTX) (Chang & Lee, 1963; Lee, Chang, Kau & Shing-Hui Luh, 1972), as well as Cobra venom (Chang & Lee, 1963; Lee & Chang, 1966; Meldrum, 1965; Lester, 1970; Eaken, Manvis & Thesleff, 1971; Earl & Excell, 1972) have been shown to produce a post-junctional blockade at skeletal neuromuscular junctions which resembles that of curare. These toxins are known to bind specifically to nicotinic receptors for ACh in electric tissue of fish (Changeux, Kasai & Lee, 1970; Miledi, Molinoff & Potter, 1971; Lester, 1970) and the end-plate regions of vertebrate skeletal muscle (Chang & Lee, 1963; Bernard, Wieckowski & Chiu, 1971; Berg, Kelly, Sangent, Williamson & Hall, 1972; Lee, 1972; Berg & Hall, 1975; Lee, Tseng & Chiu, 1967; Fertuck, Woodward & Salpeter, 1975; Fambrough & Hartzell, 1972). This highly specific binding has permitted the toxins, particularly α-bungarotoxin, to be used to localize nicotinic receptors in

skeletal muscle. Radiolabelled toxin and radioautography (Lee *et al.* 1967; Fertuck & Salpeter, 1974; Fertuck *et al.* 1975; Fambrough & Hartzell, 1972) or fluorescein-labelled toxin (Anderson & Cohen, 1974) have been used for this purpose.

The present study was undertaken to determine whether snake neurotoxins bind specifically to the ganglionic nicotinic receptors of mammals. If these toxins reach, but do not bind to ganglionic nicotinic receptors, the observation would support the hypothesis that ganglionic nicotinic receptors are different from those of the skeletal neuromuscular junction. We determined the action of α-bungarotoxin and cobra neurotoxins from *Naja naja siamensis* and *Naja nivea* on test preparations of mammalian ganglia. If the toxins bind to nicotinic receptors specifically, then they should: (*a*) block ganglionic responses to nicotine and (*b*) block ganglionic responses to preganglionic nerve stimulation. In addition, responses to post-ganglionic nerve stimulation should not be antagonized. Several preparations were used: isolated longitudinal muscle with attached myenteric plexus dissected from guinea-pig ileum; rabbit small intestine with sympathetic nerves attached; guinea-pig stomach with attached vagus nerves and the guinea-pig hypogastric nerve–vas deferens. Additional tests were done with intestine from new-born rabbit and chick embryo. Since the toxins failed to influence nicotinic responses of ganglia in any of the preparations, electron microscopic studies were done on intestinal ganglia using tracer molecules to determine whether a barrier exists which might prevent the toxins from gaining access to ganglionic receptors. No such barrier was found.

METHODS

Preparations. Guinea-pigs and rabbits were stunned by a blow on the head and exsanguinated. The small intestine was removed, cleaned, and placed in Krebs solution. The longitudinal muscle with attached myenteric plexus was dissected from the guinea-pig ileum. The isolated preparation was drawn through a pair of insulated platinum wire ring electrodes and suspended in a 10 ml. organ bath containing oxygenated Krebs solution at 37° C. The Krebs solution used in every experiment had the following composition (mM): NaCl, 133; KCl, 4·7; NaH_2PO_4, 1·3; $NaHCO_3$, 16; $CaCl_2$, 2·7; $MgCl_2$, 0·17; dextrose, 7·07.

The electrodes permitted electrical field stimulation of the preparation. When this is done with rectangular pulses of short duration, less than 0·3 msec, post-ganglionic elements of the myenteric plexus are activated but there is no direct effect of the electrical stimulation on the smooth muscle (Paton, 1955; Gershon, 1967; Paton & Zar, 1968; Paton & Vizi, 1969).

Segments of rabbit jejunum were removed with their mesenteric vasculature intact. The preparation was tied on to a J-shaped tube containing a platinum wire electrode which extended into the lumen of gut. A stainless steel screen placed next to the intestine in the bath served as a second electrode. The attached mesenteric artery was pulled through an insulated pair of platinum ring electrodes. This arrangement permitted the rabbit jejunum to be subjected to electrical field

stimulation delivered transmurally or to sympathetic nerve stimulation delivered perivascularly (Finkleman, 1930).

The stomach and oesophagus with attached vagus nerves were removed from guinea-pigs. The vagi were dissected away from the oesophagus but were left attached to the stomach. The oesophagus was tied at the cardia and the portion above the tie was cut away. The stomach was cannulated through the pylorus; the lumen was washed and filled with saline. The stomach, with attached vagus nerves, was isolated in a 100 ml. organ bath and intraluminal pressure was recorded (Paton & Vane, 1963; Campbell, 1966; Bülbring & Gershon, 1967). Vagus nerves were drawn through insulated platinum ring electrodes for stimulation with rectangular pulses of 0·1 msec at 10–30 Hz.

Vasa deferentia with attached hypogastric nerves were removed from guinea-pigs (Hukovic, 1961), and mounted in a 25 ml. organ bath. The vas deferens was drawn through a pair of platinum ring electrodes for electrical field stimulation. The hypogastric nerve was drawn through a second pair of platinum ring electrodes. Electrical field stimulation or hypogastric nerve stimulation were delivered in trains of rectangular pulses of 0·1 msec at 10–30 Hz.

Other preparations included adult mouse and new-born rabbit small intestine, and small intestine from 18-day chick embryos. These preparations were studied *in vitro* as in the case of the adult preparations described above. Electrodes were placed to permit electrical field stimulation. Preparations of α-bungarotoxin were assayed for potency by testing *in vitro* on the guinea-pig diaphragm, stimulated through the phrenic nerve (Bülbring, 1946). In some experiments animals were given α-bungarotoxin *in vivo*. Guinea-pigs were anaesthetized with pentobarbital, their abdomens were opened, and α-bungarotoxin (6 mg/kg) was injected directly into the abdominal aorta. Mice were given a lethal dose of α-bungarotoxin (6 mg/kg) intravenously, and the small intestine was removed as soon as the animals stopped breathing.

Drugs and toxins. α-Bungarotoxin was obtained from the Miami Serpentarium and purified (Lee *et al.* 1972). Toxins from *Naja naja siamensis* (unlabelled and tritium-labelled) and *Naja nivea* were obtained from Dr E. Reich and A. Maelicke at The Rockefeller University. These toxins were 99 % pure (Cooper & Reich, 1972). Drugs used were acetylcholine chloride (ACh), dimethylphenylpiperazinium iodide (DMPP), nicotine hydrogen tartrate, hyoscine hydrobromide, D-tubocurarine chloride and tetrodotoxin. Rhodamine-labelled α-bungarotoxin was prepared by conjugating α-bungarotoxin with tetramethylrhodamine isothiocyanate (TRITC) according to the method of Anderson & Cohen (1974). Essentially, α-bungarotoxin and TRITC were coupled in 0·05 M sodium carbonate buffer at pH 9·5 overnight at 4° C and then re-equilibrated with 0·05 M sodium carbonate buffer at pH 7·0. Dye–toxin conjugate was separated from unbound TRITC by passage through a column (30 × 1·5 cm) of Sephadex G-25 at 4° C.

Microscopy. Tissues were removed from both mice and guinea-pigs for electron microscopy. Small pieces of whole mouse ileum (about 1 mm^3) or of the isolated longitudinal muscle with adherent myenteric plexus dissected from the guinea-pig ileum were fixed by immersion in a solution containing 5 % glutaraldehyde, 4 % formaldehyde (generated from paraformaldehyde), and 10^{-3} M-$CaCl_2$ in 0·08 M sodium cacodylate buffer at pH 7·1–7·2 (Karnovsky, 1965). Tissues were washed overnight in buffer and post-fixed in 2 % OsO_4 in the same buffer. Some tissues were stained *en bloc* with a 2 % solution of uranyl acetate in sodium maleate buffer at 2–4° C at pH 6·2 (Karnovsky, 1967). All tissues were rapidly dehydrated through a graded series of ethanols, cleared with propylene oxide, and embedded in Epon 812. Thin sections were cut on a Porter–Blum MT 2B ultramicrotome, stained with lead citrate and uranyl acetate, and examined in a JEM 100B electron microscope.

Penetration of molecules into the myenteric plexus was assessed using lanthanum ion and ruthenium red as tracers as follows: a 2 % solution of lanthanum nitrate in 0·05 M Tris-hydrochloride buffer, pH 7·2, was added to an equal volume of aldehyde fixative which was prepared so that the final concentrations of aldehydes and buffer were equal to those stated above (Revel & Karnovsky, 1967). Tissues were fixed in this solution and subsequently post-fixed in 2 % osmium tetroxide dissolved in sodium cacodylate buffer containing 1 % lanthanum nitrate, dehydrated in a series of ethanols containing 1 % lanthanum nitrate and embedded Epon 812. These tissues were viewed without further staining. A solution of ruthenium red (1500 parts/10^6) was prepared in 3·6 % cacodylate buffered glutaraldehyde (Luft, 1971). After fixation in this solution tissues were post-fixed in 1·25 % cacodylate buffered osmium tetroxide containing ruthenium red for 3 hr before being dehydrated and embedded in Epon 812. Sections were viewed without further staining.

Radioautography was performed on 1 μm Epon sections of longitudinal muscle–myenteric plexus or oesophagus of guinea-pigs which had been incubated for 60 min with tritiated venom of *Naja naja siamensis* (6 ci/m. mole). Ilford L4 emulsion was applied by dipping as described previously (Gershon & Ross, 1966).

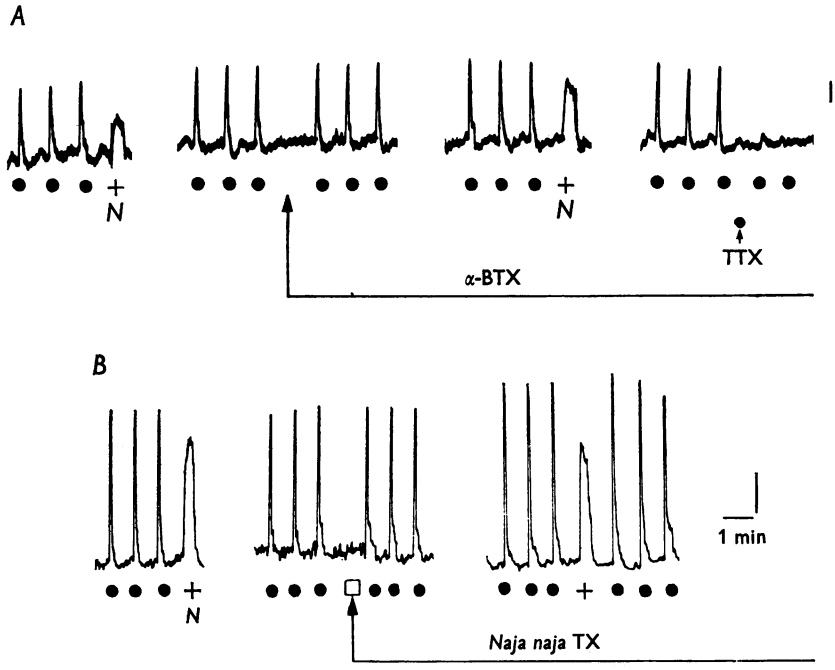
RESULTS

Longitudinal muscle–myenteric plexus of guinea-pig

The effects of α-bungarotoxin and the toxins of *Naja naja siamensis* and *Naja nivea* were tested on the longitudinal muscle–myenteric plexus of the guinea-pig. Typical responses elicited by electrical field stimulation and by the addition of nicotine to the suspending medium are shown in Text-fig. 1*A* and 1*B*. The effects of both electrical field stimulation and nicotine are mediated by cholinergic post-ganglionic neurones (Gershon 1967; Paton & Zar, 1968; Paton & Vizi, 1969). Therefore, these responses were abolished by hyoscine (10^{-7} M) or tetrodotoxin (10^{-7} g/ml.; Text-fig. 1*A*). The addition of neurotoxins to the bath did not affect responses to electrical field stimulation. Characteristic contractile responses of the same magnitude were observed before and after the addition of the neurotoxins (1·3 μM). Similarly, the responses of the longitudinal muscle–myenteric plexus to nicotine (10 μm), applied 30 min after application of toxins (Text-fig. 1*A* and *B*), were also comparable to the contractile responses to nicotine obtained before application of the toxins. Similar results were obtained employing various concentrations of the toxins (from 0·1 to 100 μM). Incubating the preparation with neurotoxins (100 μM) for as long as 4 hr failed to antagonize responses of the longitudinal muscle–myenteric plexus to electrical field stimulation or to nicotine (10 μM). As would be expected from these observations, the neurotoxins did not inhibit responses of the longitudinal muscle—myenteric plexus to ACh (1–10 nM).

Because these results were unexpected, the activity of α-bungarotoxin was tested on isolated guinea-pig diaphragm stimulated by way of the phrenic nerve. Contractions of this skeletal muscle in response to nerve

stimulation were abolished within 20 min by the same batches of α-bungarotoxin (0·1 μM) that were ineffective on the longitudinal muscle–myenteric plexus. Moreover, responses of the longitudinal muscle–myenteric plexus to nicotine were reversibly blocked by classical ganglion blocking agents such as hexamethonium (10^{-5} M) or tubocurarine (28 μM). The neurotoxins were as ineffective against the nicotinic agonists, DMPP (Paton & Zaimis, 1949), as they were against nicotine itself.

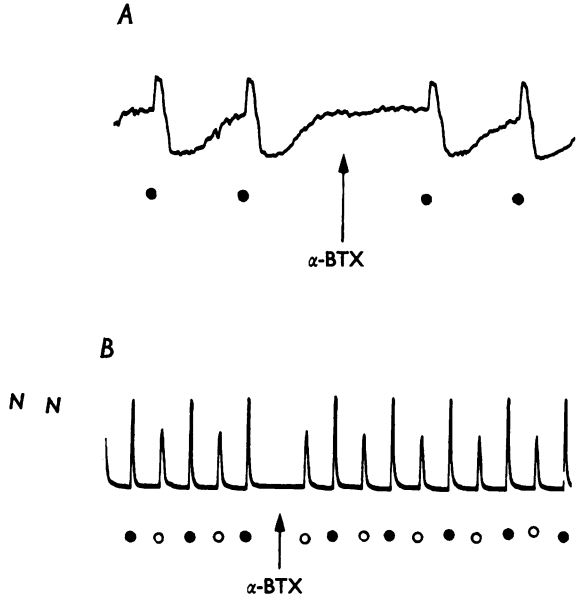


Text-fig. 1. Contraction of the longitudinal muscle with attached myenteric plexus isolated from guinea-pig ileum. *A*, contractions are elicited by electrical field stimulation (filled circles) and nicotine (*N*; 10 μM). α-bungarotoxin (α-BTX; 1·3 μM) was added at the arrow. Contractions in response to electrical field stimulation are abolished by tetrodotoxin (TTX). *B*, venom from *Naja naja siamensis* has no effect on responses to electrical field stimulation (filled circles) or nicotine (10 μM). The vertical line equals 1 mm.

Vagus nerve–stomach. The effect of the toxins on ganglionic transmission was further evaluated by determining their effect on responses of the stomach to stimulation of the vagus nerves. The guinea-pig vagus nerves contain axons presynaptic to both cholinergic excitatory and non-adrenergic intrinsic inhibitory ganglion cells (Campbell, 1966; Bülbring & Gershon, 1967). The contractile phase of the response of the stomach to

vagal stimulation is mediated by the former and the relaxant phase by the latter. Neither the contractile, nor the relaxant component of the stomach's response to stimulation of the vagi were effected by α-bungarotoxin (Text-fig. 2*A*).

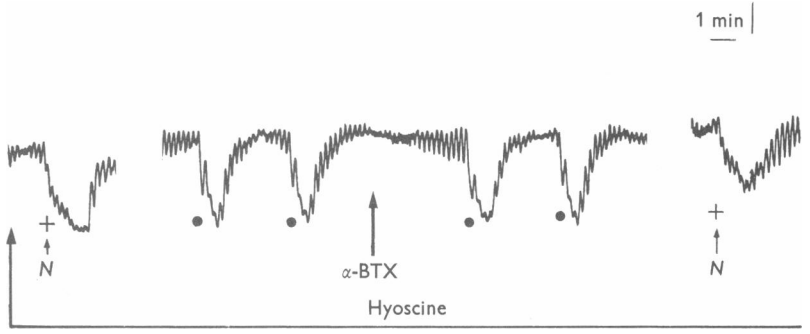
Hypogastric nerve vas deferens. Similar findings were also obtained in systems where the post-ganglionic neurone is adrenergic. The guinea-pig vas deferens was stimulated either by electrical field stimulation, activating post-ganglionic sympathetic axones directly, or by stimulation of the preganglionic axones in the hypogastric nerve. α-Bungarotoxin did not affect the responses of the vas deferens to either (Text-fig. 2*B*).



Text-fig. 2. *A*, mechanical responses of the isolated guinea-pig stomach to vagal stimulation (filled circles). There is no antagonism of either contractile or relaxant components of the response by α-bungarotoxin (1·3 μM). *B*, contractions of the isolated guinea-pig vas deferens in response to electrical field stimulation (filled circles) and to stimulation via the hypogastric nerves (open circles). α-Bungarotoxin (1·3 μM) has no effect on either. The vertical line equals 1 mm.

Rabbit small intestine. The evidence from guinea-pig stomach that neurotoxins do not affect non-adrenergic inhibitory ganglion cells was supported by observations of rabbit small intestine treated with hyoscine (0·1 μM). The addition of this muscarinic antagonist blocks the action of the excitatory transmitter, ACh, and thus the action of intrinsic inhibitory neurones is unmasked by the drug. These intrinsic inhibitory

neurones have nicotinic receptors and are stimulated by nicotine (Gershon, 1967; Bülbring & Gershon, 1967). In the presence of hyoscine, the previously contractile effect of nicotine and electrical field stimulation is reversed and nicotine and electrical field stimulation now relax the gut (Text-fig. 3). Since these relaxations are of neural origin, they are abolished by tetrodotoxin (Gershon, 1967). α-bungarotoxin neither antagonized the relaxations elicited by nicotine nor those elicited by electrical field stimulation. Therefore, the intrinsic intestinal inhibitory neurones also are unaffected by α-bungarotoxin.



Text-fig. 3. Non-adrenergic relaxant responses of the rabbit jejunum to nicotine (10 μM) and electrical field stimulation (filled circles). Hyoscine (10^{-7} M) was present throughout. There is no antagonism by α-bungarotoxin (13 μM). The vertical line equals 1 mm.

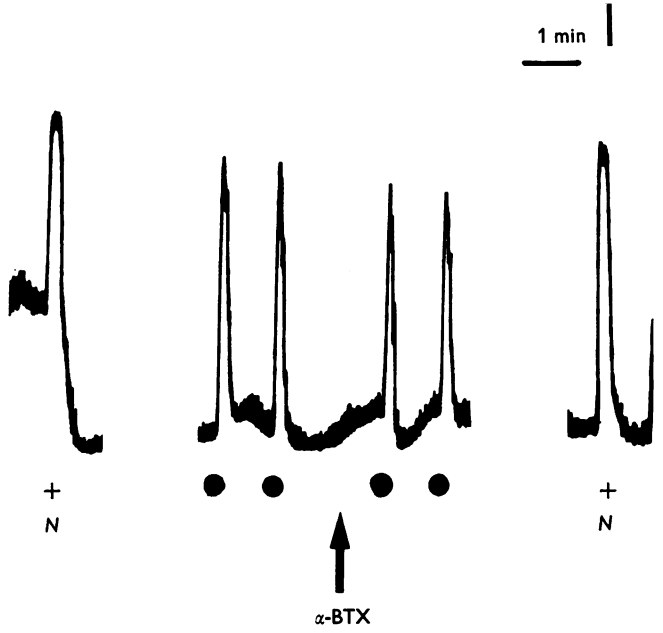
Toxin penetration

Snake neurotoxins thus failed to influence virtually any of the neural parameters we studied. These results could be explained if: (*a*) ganglionic nicotinic receptors are different from those of skeletal neuromuscular junctions; or (*b*) the toxins fail, because of a permeability barrier, to reach the ganglionic receptors. We tried to circumvent potential permeability barriers by testing the effect of α-bungarotoxin on the intestine of the new-born rabbit or the 18-day chick embryo, when such a barrier might not yet have developed. Both preparations contracted in response to nicotine (10 μM) but this response was not blocked by α-bungarotoxin (Text-fig. 4).

Labelled neurotoxins. We used labelled venoms in order to see if binding of toxins could be detected in ganglia of the myenteric plexus. Dissected strips of longitudinal muscle with attached myenteric plexus from guinea-pigs were incubated for 1 hr either with rhodamine-labelled α-bungarotoxin or tritium-labelled toxin from *Naja naja siamensis*. The striated muscle of guinea-pig oesophagus and diaphragm containing neuromuscular junctions was similarly exposed to the labelled toxins and served as a

control. No restriction of either label to any recognizable structure was found in the myenteric plexus by fluorescence microscopy (α-bungarotoxin) or autoradiography (*Naja naja siamensis*). In contrast, labelling of neuromuscular junctions in skeletal muscle was readily seen (Pl. 1*A*).

Electron microscopy. We used electron microscopy to search for the presence of a possible permeability barrier in the myenteric plexus. Lanthanum and ruthenium red were used as tracer molecules to facilitate this search. Lanthanum, a cation like the neurotoxins, has a Stokes–Einstein radius of 2·8 nm and so is somewhat larger than α-bungarotoxin whose Stokes–Einstein radius is about 2·0 nm (A. Maelicke, personal communication).



Text-fig. 4. Mechanical responses of the jejunum of a new-born rabbit to electrical field stimulation (filled circles) and nicotine (10 μM). Neither is antagonized by α-bungarotoxin (1·3 μM). The vertical line equals 1 mm.

The structure of the myenteric plexus does not suggest that modifications exist which might prevent macromolecules from gaining access to the interior of the plexus. As is characteristic of the autonomic nervous system ,axon terminals abut directly on the connective tissue space (Pl. 1*B*). Schwann processes only partially enclose the plexus and leave considerable open space between axons (Pl. 1*B*). Blood vessels lie in the muscle layer and do not enter the plexus. In ganglionic regions of the

plexus, dendrites and even ganglion cell somata, uncovered by satellite cells reach the connective tissue space.

When lanthanum nitrate is included in the fixative and subsequent processing solutions, the tracer readily penetrates into the intercellular space between axones (Pl. 2*A*). Lanthanum does not occur intracellularly except in occasional axon terminals where it is found in synaptic vesicles (Pl. 2*A* and inset). Lanthanum also reaches ganglion cells and is found underlying preganglionic axon terminals within synaptic gaps. Like lanthanum, ruthenium red also penetrates into the myenteric plexus and is found between axons and Schwann processes, and around ganglion cells and dendrites. Thus, both tracer molecules, lanthanum and ruthenium red, reach all parts of the myenteric plexus.

DISCUSSION

Our experiments have failed to demonstrate any effect of the snake neurotoxins, α-bungarotoxin or venoms from *Naja naja siamensis* or *Naja nivea*, on ganglionic nicotinic receptors. The toxins failed to antagonize nicotinic responses no matter whether the post-ganglionic element was cholinergic (myenteric plexus), adrenergic (vas deferens) or other (non-adrenergic inhibitory). Moreover, labelled toxins also failed to bind to structures in the ganglia of the myenteric plexus.

Our data indicated that no protective barrier sufficient to exclude the neurotoxins exists. New-born or even embryonic tissue was as refractory to toxin blockade as was adult tissue. More importantly, ultrastructural evidence indicated that there is no barrier in the myenteric ganglia. The neural tissue is incompletely ensheathed and is open to the connective tissue space at axone terminals. Even ganglion cells and dendrites are bare to the connective tissue in many regions. These structures must have been reached by the toxins. Moreover, tracer molecules such as lanthanum ion and ruthenium red readily penetrate into all parts of the myenteric plexus even reaching synaptic gaps. Lanthanum ion resembles α-bungarotoxin in carrying a positive charge and is actually larger. Since lanthanum penetrates into the plexus it seems reasonable to conclude that the snake neurotoxins penetrate as well.

If the toxins do reach the ganglionic nicotinic receptors, and it seems likely that this is the case, then their failure to act on these receptors must be due to differences from those of the skeletal muscle in the molecular structure of the receptors themselves. The receptor of the skeletal neuromuscular junction thus represents only one type of nicotinic ACh receptor. The receptors of fish electric organs are similar to these both in sensitivity to α-bungarotoxin (Changeux *et al.* 1970; Miledi *et al.* 1971; Lester, 1970)

and immunological reactivity (Daniels & Vogel, 1975). However, other ACh receptors exist. For example, there are three different receptors for ACh in *Aplysia* which produce conductance changes mediated by Na^+, by K^+, or by Cl^- respectively (Gershenfield, 1972). α-BTX effects only one of these, the fast inhibitory post-synaptic potential (i.p.s.p.) associated with chloride conductance changes (Kehoe, Selock & Bon, 1976). Even here, there are differences from the skeletal neuromuscular junction in that the effect of α-bungarotoxin is reversible in *Aplysia* and the i.p.s.p. is partially blocked by atropine. The ACh receptor of *Aplysia* which leads to an excitatory post-synaptic potential (e.p.s.p.) through conductance changes mediated by Na^+ is most like the vertebrate ganglionic receptor in that it is sensitive to blockade by hexamethonium (Gershenfield, 1972; Kandel, 1976). This receptor in *Aplysia*, like that of vertebrates is unaffected by α-bungarotoxin. Muscarinic receptors, of course, are not affected by α-bungarotoxin but our data indicate that subtypes of nicotinic receptors are also present in the vertebrate nervous system which are also resistant to this agent.

In 1973, Greene, Sytkowski, Vogel & Nirenberg, reported that ^{125}I-labelled α-bungarotoxin bound to dissociated chick ganglion cells growing in culture. This binding could be antagonized by some nicotinic receptor antagonists, such as D-tubocurarine and nicotine. However, hexamethonium was a poor antagonist and was little more potent than the muscarinic antagonist atropine, in preventing toxin binding. On the basis of these observations, they suggested that α-bungarotoxin could bind to ganglionic nicotinic receptors as well as those of the neuromuscular junction. A disquieting inconsistency with this suggestion is the relative impotency of hexamethonium. Ganglionic receptors should be differentially sensitive to this agent (Paton & Zaimis, 1949). No physiological studies were done to demonstrate the identity between the toxin binding sites and actual nicotinic receptors. There have also been reports of α-bungarotoxin binding to tissue derived from the mammalian central nervous system (Moore & Loy, 1972; Bosmann, 1972). There is evidence that many of the binding sites for α-bungarotoxin in brain are not on ACh receptors (Schleifer & Eldefrawi, 1974). In the light of our observations on the nicotinic receptors of ganglion cells and observations supported by the experiments of others on cultured ganglion cells (Rees, Bunge, Barlett & Bunge, 1976; Nurse & O'Lague, 1975), these reports of α-bungarotoxin binding should be interpreted with caution. Physiological evidence should be obtained indicating that nicotinic receptors in the central nervous system are like those of skeletal muscle rather than those of ganglia. Before this evidence is available it is premature to equate α-bungarotoxin binding in the nervous system, if not in muscle, with the identification of ACh

receptors. Moreover, our data indicate that absence of α-bungarotoxin binding cannot be taken as evidence for absence of ACh receptors.

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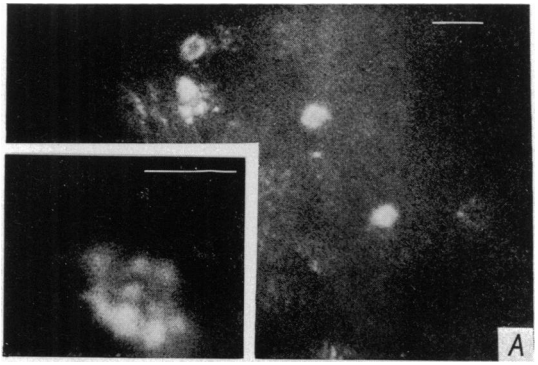
EXPLANATION OF PLATES

PLATE 1

A, fluorescence of neuromuscular junctions of guinea-pig oesophagus and (inset) diaphragm incubated for 60 min with α-bungarotoxin–TRITC. The washed tissue was teased apart and the striated muscle cells, suspended in buffer, were put on to glass slides and cover-slipped. The cells were viewed with a Leitz microscope equipped for incident light fluorescence. Light was directed through a TK580 dichroic mirror and a K580 suppression filter. The bar represents 30 μm and, in the inset, 10 μm.

B, electron micrograph showing a portion of the circular muscle and the myenteric plexus from guinea-pig intestine. Terminal varicosities (*V*) abut directly on the connective tissue space (arrows). The ensheathment by Schwann cell processes (*S*) is incomplete. The bar represents 1 μm.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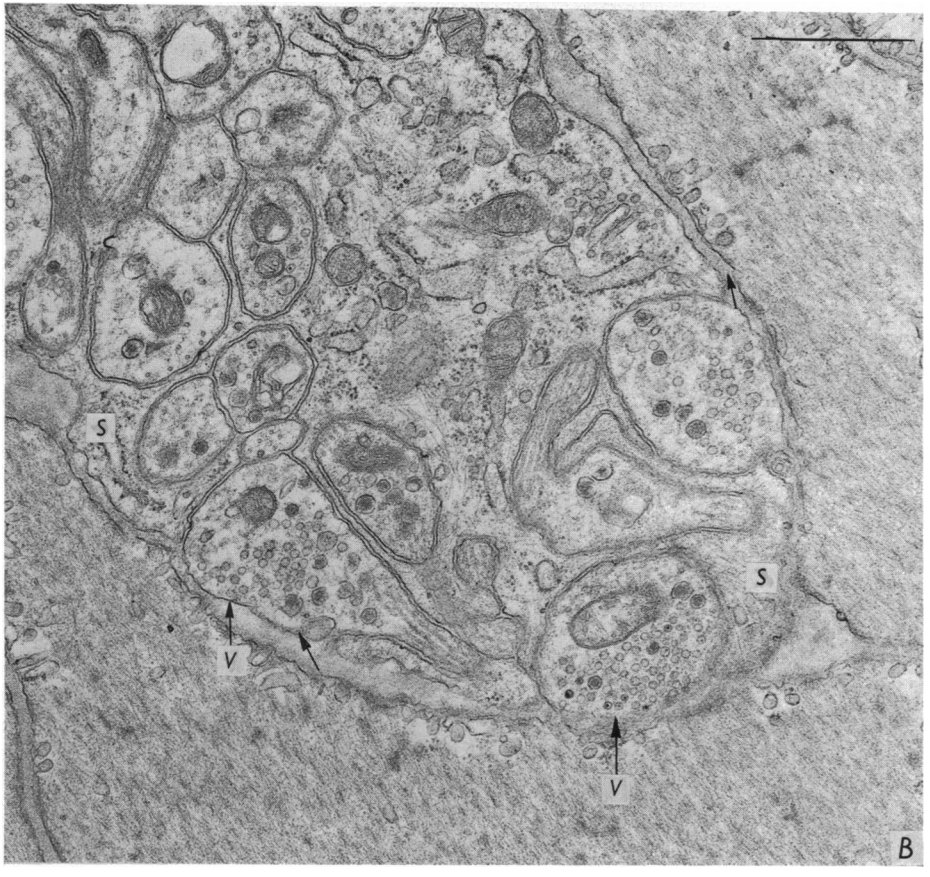
S
S
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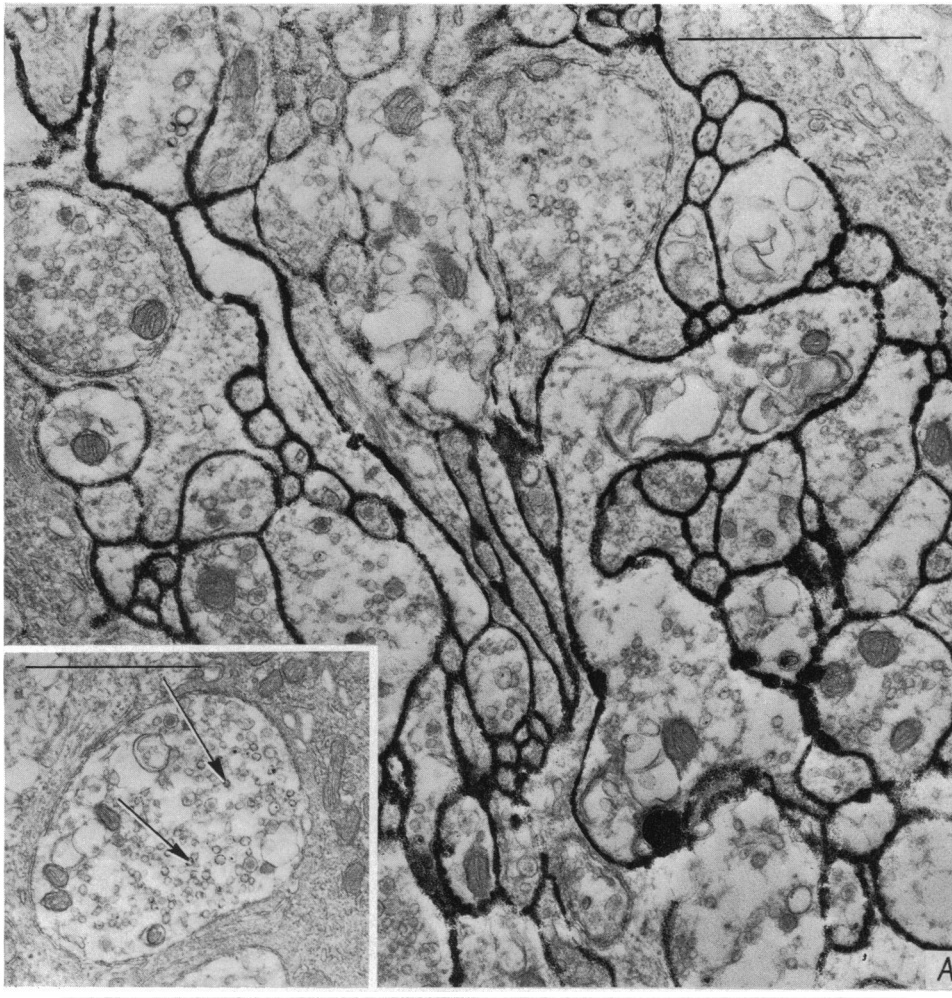
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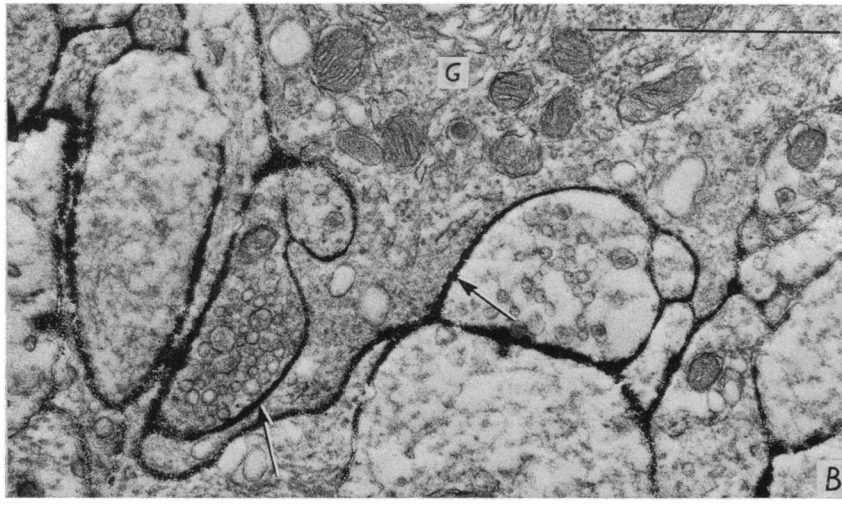
G
B

PLATE 2

A, a portion of the myenteric plexus exposed to a lanthanum containing fixative. The tracer fills the intercellular space. Some lanthanum has gained entrance to synaptic vesicles (arrow; see inset). The bar represents 1 μm.

B, lanthanum has reached the perikaryon of a ganglion cell (*G*) and appears in synaptic gap (arrows). The bar represents 1 μm.