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AN INVESTIGATION OF THE ROLE OF GANGLIA IN THE INNERVATION OF THE RAT ANOCOCCYGEUS MUSCLE: AN ELECTRICAL AND MECHANICAL STUDY

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1 A preparation is described which allows the rat anococcygeus muscle to be stimulated *via* its two extrinsic nerves. Each nerve contains both excitatory and inhibitory fibres. A ganglionated nerve plexus lies on the surface of the muscle.

2 The possibility that at least part of the excitatory pathway was interrupted by a ganglion synapse lying in one of the nodes of plexus close to the muscle was suggested by the observations that (a) the excitatory response to extrinsic nerve stimulation was reduced by the nicotinic antagonists tubocurarine (0.13 to 0.26 mM) and dihydro β-erythroidine (0.1 to 0.14 mM). (b) Fibres from one extrinsic nerve were shown to synapse on a ganglion cell from which intracellular recordings were made while the output from this ganglion cell was traced microscopically to the muscle.

3 Intracellular recording from ganglion cells in this plexus indicated that cholinergic synaptic transmission occurred in these ganglia. Tubocurarine (0.13 mM) and hexamethonium (1.3 mM) reversibly abolished intracellularly-recorded synaptic potentials.

4 Hexamethonium (0.1 to 1 mM) initially enhanced the motor response to nerve stimulation and raised muscle tone, probably by an action involving pre- and postsynaptic sites. Subsequently, hexamethonium inhibited the response to extrinsic nerve stimulation presumably by an effect at ganglia lying along the excitatory pathway. Hexamethonium enhanced, without subsequently inhibiting, the response to exogenously added noradrenaline in both untreated and 6-hydroxydopamine-treated rats. These results suggest that the initial enhancement produced by hexamethonium involved sites at postganglionic nerve endings and on smooth muscle receptors.

5 Inhibitory responses were obtained following extrinsic nerve stimulation when the tone of the muscle was raised and the excitatory response abolished by either guanethidine (3 μM) alone or by carbachol (10 μM) followed by phentolamine (3 μM). The inhibitory response was not reduced by hexamethonium (up to 2.8 mM) tubocurarine (up to 1.3 mM) or by atropine (up to 1 μM).

Introduction

The rat anococcygeus muscle (Gillespie, 1972; Creed, Gillespie & Muir, 1975) possesses both a motor (adrenergic) and an inhibitory innervation. The nature of the inhibitory transmitter remains unknown. Histological studies have revealed a ganglion, containing some 50 cells lying in the vicinity of the muscle (Gillespie & Lüllman-Rauch, 1974). No role for this ganglion in the innervation of the anococcygeus muscle has been put forward since hexamethonium (C_6) did not inhibit contractions of the muscle in response to preganglionic nerve stimulation (Gillespie, 1972). In the course of a subsequent investigation (McKirdy & Muir, 1976a), it became clear that many ganglia were located on the complex pathways which innervate the anococcygeus muscle. Because of the obvious antomical proximity of these ganglia to the muscle, a re-investigation of their role in the innervation of the muscle was undertaken as part of the present study. In contrast to previous findings, the present results suggest that at least some of the ganglionic neurones may play a role in the innervation of the muscle.

In the second part of the investigation, an analysis of the transmission processes at the ganglion was made by means of intracellular recording techniques. There were three objectives in mind: (a) to confirm

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that synaptic transmission takes place; (b) to compare the transmission process and the action of ganglion blocking agents thereon with that at other pelvic ganglia (Holman, Muir, Szurszewski & Yonemura, 1971) and (c) to determine whether or not the inhibitory response of the muscle could have arisen from an inhibition of the excitatory mechanisms at the ganglion synapse. Such an inhibition could be produced by catecholamines released, for example, from the small intensely fluorescent (SIF) cells known to exist (Boyle, McKirdy & Muir, 1977) in these ganglia. Preliminary accounts of this work have already been published (McKirdy & Muir, 1976a, b).

Methods

Anococcygeus muscles were removed from male Wistar rats (200 to 350 g) as described by Gillespie (1972). The occurrence and position of the ganglia were examined in spread preparations stained with gold chloride (Gairns, 1930). The muscle with surrounding tissue, including the extrinsic nerves, was pinned out (Figure 1) and a region of nerve plexus suspected of containing ganglion cells removed from it and pinned out on Sylgard (Dow Corning).

To investigate the role of the ganglia in the innervation of the anococcygeus muscle two preparations were studied. (a) The anococcygeus muscle with intact extrinsic nerve supply was set up in a bath (Figure 3) and perfused with Krebs solution (for composition see Creed *et al.*, 1975) at 37°C. Each nerve, one a branch of the external spermatic division of the genitofemoral and the other (which was usually accompanied by a blood vessel) a branch of the posterior scrotal division of the perineal nerve, was drawn into a small suction electrode positioned 0.5 to 1 cm from the muscle. In about a quarter of the preparations, a third nerve (Figure 1) was observed and, when present, was taken up with the fibres from the genitofemoral nerve. One end of the muscle was fixed and the other was connected via a lever system to an isometric strain gauge and a Grass Polygraph to measure tension. (b) The anococcygeus muscle was isolated without the extrinsic nerve supply and associated ganglia and prepared for field stimulation of the intramural nerves as previously described (Gillespie, 1972). Tension was measured with an isometric transducer and displayed on a Devices recorder.

To examine transmission processes, a region of nerve plexus suspected of containing ganglion cells was pinned out on Sylgard on a cover slip which formed the base of a bath. The bath was mounted on the stage of an inverted microscope and the ganglion-containing plexus super-perfused with Krebs solution at 27°C. Intracellular recordings were made from ganglion cells by a technique similar to that described by Hirst, Holman & Spence (1974). A microelectrode (80 to 180 MΩ) filled with 2 M KCl was manoeuvred over a ganglion cell under visual control and the cell impaled. A bridge-circuit incorporated into the preamplifier (W.P.I.), allowed current to be passed through the recording microelectrode. Following impalement of a neurone, its response to focal electrical stimulation (Nishi & North, 1973) of associated nerve fibre tracts by a second microelectrode (tip diameter 5 to 20 μm, filled with normal saline) was examined; the stimulating electrode was positioned 50 to 200 μm from the impaled cell.

The following drugs were used: atropine sulphate (BDH); carbachol chloride (Sigma); 6-hydroxydopamine hydrochloride (6-OHDA, Sigma); edrophonium chloride (Roche); dihydro-β-erythroidine hydrobromide (DHBE, Merck, Sharpe & Dohme); guanethidine sulphate (Ciba); hexamethonium bromide (C_6, May & Baker); noradrenaline bitartrate (NA, Koch-Light); phentolamine mesylate (Ciba); tetrodotoxin (Sankyo); tubocurarine chloride (Tc, Burroughs Wellcome).

Results

Anatomical observations

The extrinsic nerves run towards the anal end of the muscle and divide as they approach the muscle, ramifying to form a nerve network on the lateral surface of the muscle (Figure 1a). Some nerve branches run on presumably to supply other structures. In about a quarter of the preparations, a third nerve joined this network (Figure 1a); although we have been unable to trace this nerve to its origin it appears to come down the posterior pelvic wall. Microscopic examination of nerve fibres in this network revealed the presence of ganglion cells at several of the junctions (Figure 2). Nerve fibre tracts have been observed to connect, by running through the muscle mass, the network or 'plexus' on the lateral surface with another, finer, plexus lying on the medial surface of the muscle at or near the ventral bar. Fine nerve fibre tracts leave the network on the lateral surface of the muscle and run into the mass of muscle dividing into smaller tracts as they run along the muscle bundles. In several preparations, nerve plexuses containing ganglion cells have been found within the muscle mass. These can easily be missed and often may be included in preparations of the muscle examined using field stimulation. The number of ganglion cells in each ganglion is not large and the pattern of their occurrence very variable. In most preparations however one larger ganglion was usually observed which contained approximately 50 to 70 cells.

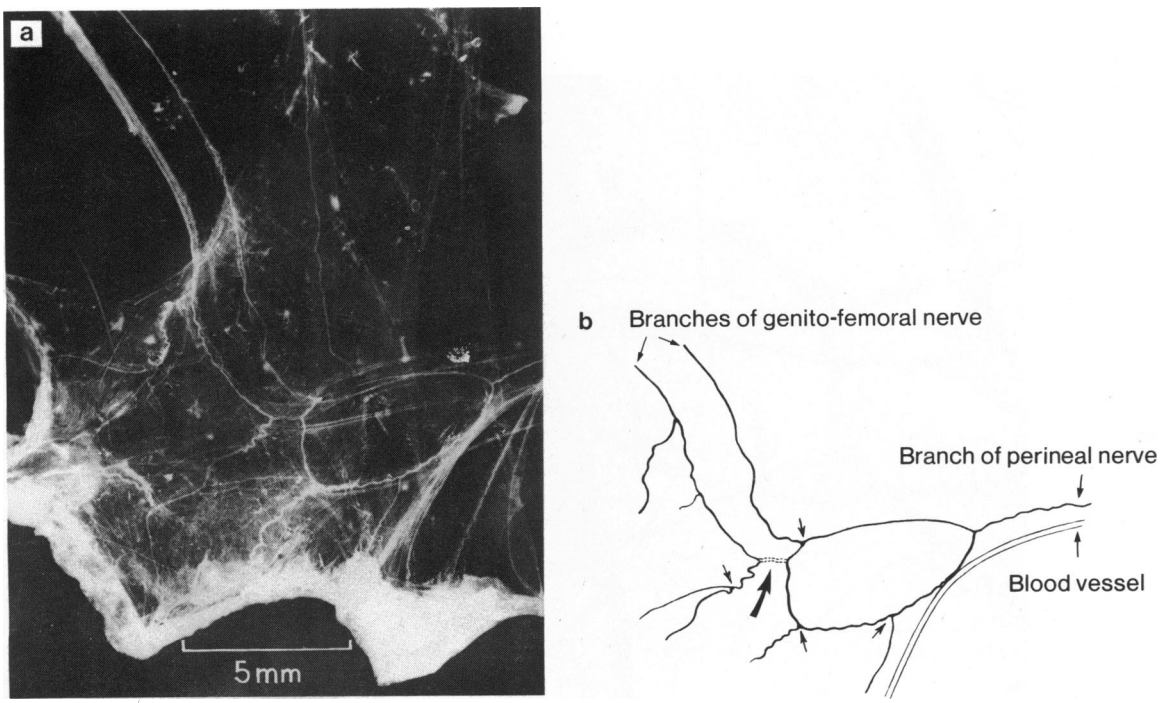

Figure 1 (a) The anococcygeus muscle of the rat and associated nerve fibre tracts (unstained). (b) Diagrammatic representation of sites of ganglia (small arrows) and associated extrinsic nerves shown in (a). The large arrow indicates the location of a particularly large ganglion found in this preparation. The two extrinsic nerves are branches of the genito-femoral nerve and of the perineal nerve. A third, inconstant nerve (not shown in b) lying between the other two extrinsic nerves in (a) was taken with fibres of the genito-femoral nerve for stimulation purposes.

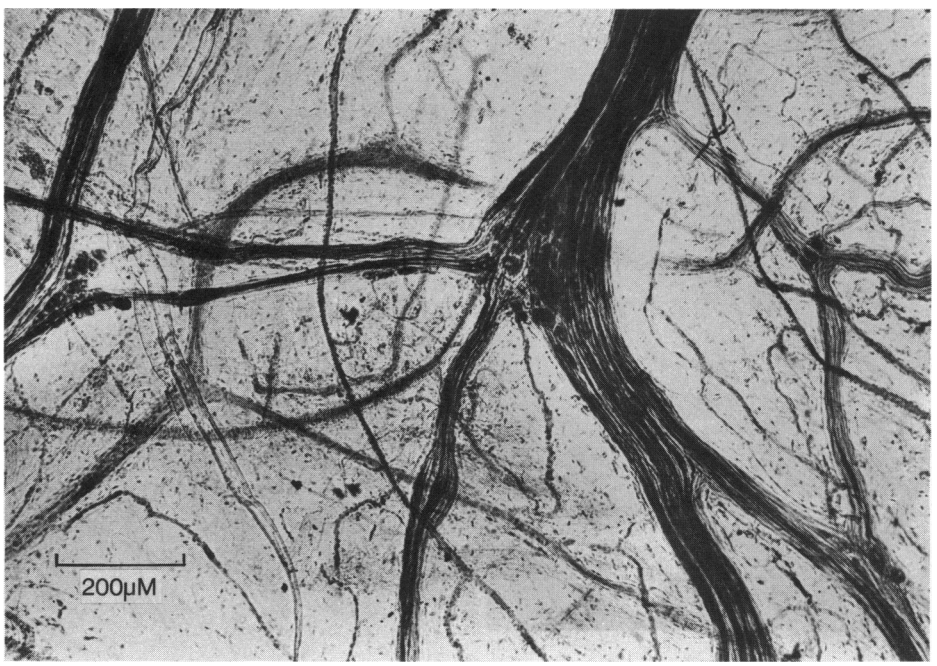

Figure 2 Rat anococcygeus muscle: part of the ganglion plexus stained for nerve tissue with gold chloride. Note the nodes of ganglion cells at several of the interconnecting fibres of the extrinsic (genito-femoral) nerve.

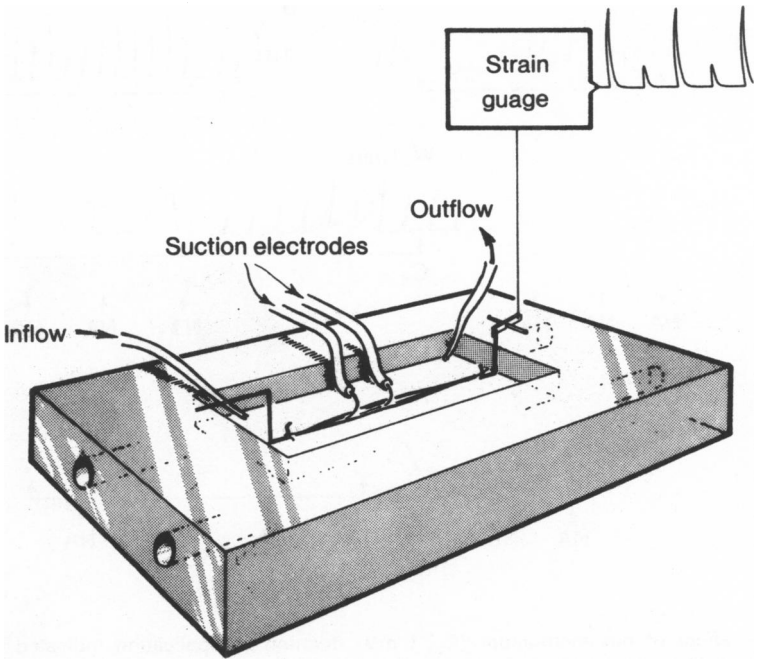

Figure 3 Drawing of bath, in which the rat anococcygeus muscle was stimulated via the extrinsic nerves by means of suction electrodes. Flow inducers (Watson Marlow) attached to the inflow and outflow regulated the level of the perfusion fluid which was maintained at 37°C by hot water pumped through the two channels cut in the solid perspex.

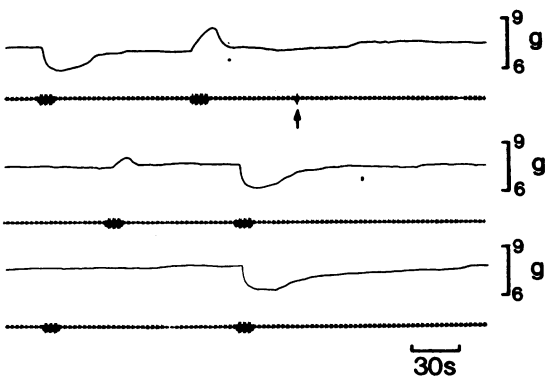

Figure 4 Continuous record from a preparation of the rat anococcygeus muscle with extrinsic nerves which developed tone without the addition of drugs. The contractions (upward deflections) were obtained in response to stimulation of the genito-femoral nerve, for the period indicated by the horizontal bars; relaxations (downward deflections) in response to stimulation of the perineal nerve. Tubocurarine (0.13 mM), added at the arrow, slightly increased tone, abolished the contractions and prolonged the relaxations produced by extrinsic nerve stimulation.

The effects of drugs on the motor response

In experiments where the muscle was set up as shown in Figure 3 a contraction was produced on stimulating (0.3 ms, supramaximal voltage) either the genito-femoral or the perineal nerve (optimal frequency about 30 Hz); the contraction that was produced on stimulating the genito-femoral nerve was usually the larger. No contraction was produced with single pulses or at frequencies below 1 to 2 Hz. The contraction produced by stimulation of either nerve was abolished by tetrodotoxin (3 μM) confirming an earlier view that it was neurally-mediated, and was inhibited by phentolamine (5 μM) showing that these nerves are adrenergic (Gillespie, 1972).

To determine the role of ganglia in the innervation of the muscle, the effects of Tc, DHBE and C_6 on the motor response to extrinsic nerve stimulation, to field stimulation and to applied NA were each examined. Two types of results were obtained. On the one hand, Tc (0.13 to 0.26 mM) or DHBE (0.1 to 0.14 mM) reduced and occasionally abolished (Figure 4) the motor response to extrinsic nerve stimulation and produced no enhancement. This inhibition was antagonized by the anticholinesterase, edrophonium

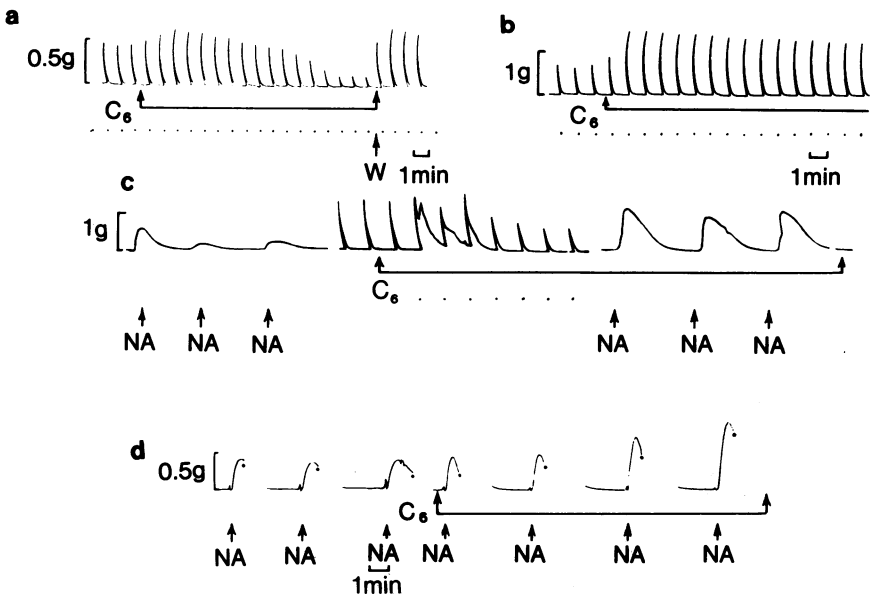

Figure 5 The effect of hexamethonium (C_6, 1 mM; duration of application indicated by a solid line) on the response of the rat anococcygeus muscle to stimulation of the genito-femoral nerve (30 Hz, 0.1 ms, supramaximal voltage), to field stimulation (30 Hz, 0.5 ms, supramaximal voltage) and to applied noradrenaline (NA). In (a) the initial enhancement of the motor response to extrinsic nerve stimulation declined in the presence of C_6 to below control values presumably due to ganglion blockade. This decline was reversible on washing (W). In contrast, the enhancement of the motor response to field stimulation (b) in the presence of C_6 was well maintained. The effect of C_6 on added NA (1 μM) and on stimulation of the genito-femoral nerve in the same experiment (c; time scale same as d) showed that C_6 initially prolonged then inhibited the nerve response. In contrast, responses to NA were persistently enhanced. In this particular experiment the time taken for C_6 to reach the preparation was approximately 80 s. Times between panels were 17 and 5 min. (d) The enhancement by C_6 of the response to NA (3 μM) in the 6-hydroxydopamine-treated animal suggests a postsynaptic mode of action.

(5 μM). Tc (0.1 mM) also reduced the response of the muscle to field stimulation on some occasions. However, the reduction was small compared to that produced when the extrinsic nerves were stimulated and arose presumably from an inhibitory action at the residual ganglia within the muscle mass. Tc (0.13 to 0.26 mM) initially depressed the response of the muscle to added NA but the response recovered in the continued presence of Tc. On the other hand, C_6 (0.1 to 1 mM) enhanced (by up to 100%) the motor response to both extrinsic nerve *and* field stimulation, to a similar extent. (Interestingly, in the rabbit rectococcygeus muscle, C_6 is more effective than Tc in reducing the excitatory response: King, McKirdy & Wai, 1977.) The enhancement was affected by the method of stimulation employed. Following extrinsic nerve stimulation (Figure 5a), the enhancement was usually short-lived and, in the continued presence of C_6, the motor response declined to below control levels. This enhancement and subsequent inhibition of the motor response were reversed on washing. In contrast, the enhancement of the motor response to field stimulation was well maintained and remained undiminished in the continued presence of C_6 for up to 75 min (Figure 5b). The enhancement by C_6 of the motor response to extrinsic nerve or to field stimulation was reduced by phentolamine (8 μM). C_6 (0.1 to 1 mM) also enhanced the response to exogenously added NA (3 μM) (Figure 5c). This latter enhancement, was prolonged and persisted until washing.

The results with exogenously-added NA suggested that C_6 was exerting a postsynaptic effect on the smooth muscle membrane. To examine this possibility further, 6-OHDA was used (2 × 0.2 mmol/kg day 1 then 2 × 0.4 mmol/kg day 4, i.p., animals killed on day 5) to destroy the adrenergic nerves. This dose has been found to eliminate the adrenergic nerves as judged by the absence of fluorescence with the Falck histochemical technique (Gibson & Gillespie, 1973).

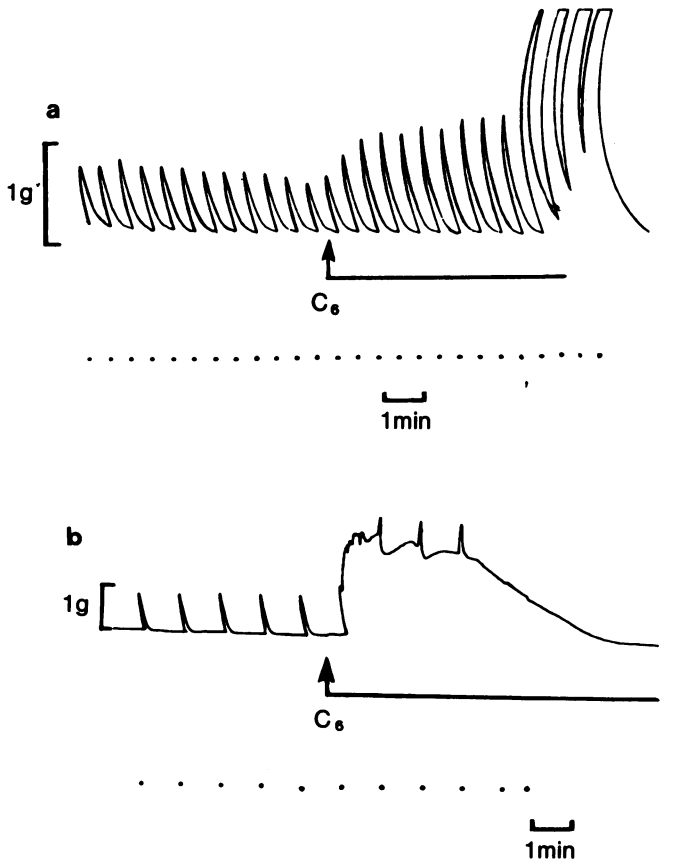

Figure 6 (a) The effect of hexamethonium (C_6, 0.3 mM solid line) on the response of the rat anococcygeus to field stimulation (20 Hz, 0.5 ms for 1 s every 30 s, supramaximal voltage). The response of the muscle to field stimulation was enhanced and the amplitude of the muscle contractions greatly increased. In (b), C_6 (0.1 mM, solid line) raised the tone of the muscle and in so doing altered the response to stimulation of the perineal nerve (30 Hz, 0.25 ms, supramaximal voltage) from a purely excitatory one to one which showed both excitatory and inhibitory components.

In preparations from animals thus pretreated (Figure 5d) C_6 still enhanced the responses to applied NA. These preparations, which (as expected) did not respond to extrinsic nerve or field stimulation, were more sensitive to NA and showed a greater degree of enhancement in the presence of C_6 than those from untreated animals.

The effect of hexamethonium on muscle tone

The contractions produced by bursts of repetitive field stimulation (20 Hz, 0.5 ms, 1 s duration) at intervals of less than 1 min showed a gradual decline. Addition of C_6 (0.3 mM) reversed this decline and produced an increase in tone on which large muscle evoked contractions were superimposed (Figure 6a). These effects were reversed by washing. In a few experiments the increase in tone in the presence of C_6, was sufficiently great for the response to nerve stimulation to show an inhibitory as well as an excitatory component (Figure 6b); a similar effect can also be induced by guanethidine in this muscle (Gillespie, 1972).

The effects of drugs on the inhibitory response

Inhibitory responses were normally visible following extrinsic nerve stimulation only when the tone of the muscle was raised and the excitatory response abolished by either guanethidine (3 μM) alone or by carbachol (10 μM) followed by phentolamine 3 μM). After either procedure, inhibitory responses appeared in

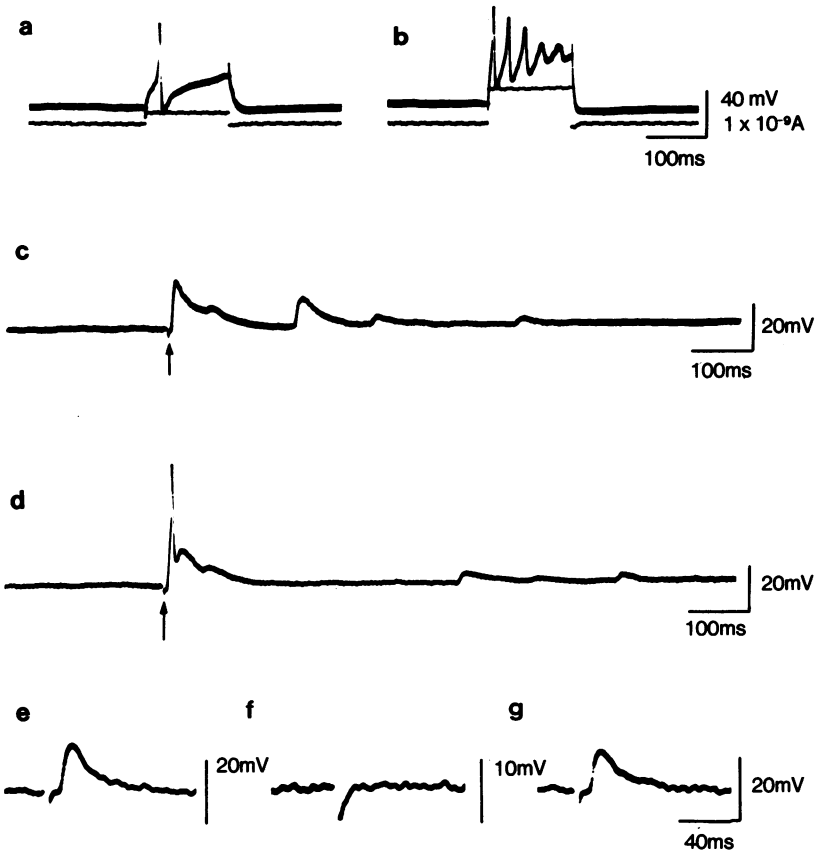

Figure 7 Responses of ganglion cells. (a) and (b) Show the effect of passing a depolarizing current pulse through the intracellular recording microelectrode to produce (a) a single action potential, (b) in the same cell following an increase in the intensity of current, a series of spikes which are rapidly 'damped out'; (c) shows, in a different cell, the miniature synaptic potentials. These occurred irregularly following a single focal stimulus (arrow) to an adjacent nerve tract. (d) Shows, in a different cell a synaptic potential following a single focal stimulus (arrow) reaching threshold to elicit an action potential. In (e–g) in a different cell, the effect on the synaptic potential (e) evoked by single pulse focal stimulation (2 ms) of adding hexamethonium (1.3 mM) to the perfusing medium for 15 min (f). The sensitivity and pulse width was increased (5 ms) in (f) but no synaptic potentials were recorded. (g) Shows the almost complete recovery 20 min after washing out hexamethonium (pulse width 2 ms).

about half of the preparations studied. (The failure to detect an inhibitory response in the remainder may have been due to nerve damage during the dissection or failure to take up the appropriate fibres into the stimulating electrode.) The inhibitory response was produced following stimulation (0.3 ms, supramaximal) of either extrinsic nerve with an optimal frequency of about 10 Hz. The inhibitory response had a variable latency of 1.5 to 5 s and was abolished by tetrodotoxin (1 μM) suggesting that it was neurally-mediated. The inhibitory response was not reduced by C_6 (up to 2.8 mM) or Tc (up to 1.3 mM) or by atropine (up to 1 μM). Indeed, in some preparations the amplitude and duration of the relaxation were increased (Figure 4) and/or the onset of the response accelerated by Tc.

Results from intracellular recording experiments

Individual ganglion cells (diameter 20 to 30 μm) were usually clearly visible but they were extremely difficult to hold with intracellular electrodes. Over 200 cells have been impaled but most were lost within a few seconds and only 26 were held long enough to examine the response to focal stimulation of fibre tracts associated with an individual ganglion. Following im-

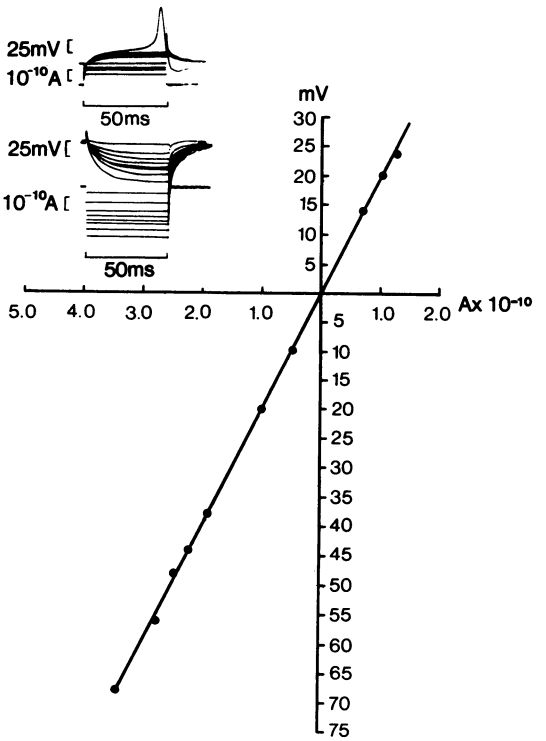

Figure 8 The response of the membrane potential to the passage of intracellular current in a typical cell in the rat anococcygeus ganglion (inset). The current-voltage relationship is shown in the graph; the first quadrant represents depolarizing and the third hyperpolarizing responses. The input resistance was approximately 190 megaohms.

palement there was no discharge of action potentials, a feature sometimes associated with intracellular penetration of other ganglionic neurones (Blackman, Crowcroft, Devine, Holman & Yonemura, 1969). It was found necessary to use high (180 to 200 MΩ) resistance microelectrodes; these had large and variable tip potentials, and no reliance has been placed on the measured (30 to 60 mV) value of the resting membrane potential (see Hirst *et al.*, 1974). Successful impalement of a neurone was indicated by the instant development of a stable membrane potential, which remained fairly constant, and the ability of a pulse of depolarizing current (1 to 10 × 10^{-10}A, 10 to 150 ms) passed through the recording microelectrode to elicit an action potential in the cell. When the strength of the depolarizing current pulse, was raised from zero to just threshold, one action potential was elicited (Figure 7a); when the current intensity was increased, a second spike was elicited in most, but not all, cells. The second spike, when present, was smaller in amplitude than the first. When the current intensity was increased further, the firing rate of those cells which showed more than one spike increased and further spikes appeared; however, this train of spikes was rapidly 'damped out' (Figure 7b). A similar pattern of activity has previously been shown for some cells in other ganglia; such behaviour could have arisen from cell damage (Blackman, personal communication) or from accommodation by the cell membrane. However, since this behaviour was observed in four cells, each of which was held for over 3 h (during which 80 to 100 mV single spikes could be elicited), the first explanation seems unlikely. Current/voltage relationships for both hyperpolarizing and depolarizing currents using long pulses (50 to 150 ms) were examined in 8 cells (Figure 8). Values of input resistance varied from 95 to 200 MΩ.

Suprathreshold pulses (10 to 30 ms) of depolarizing current were passed through the recording electrode at frequencies of from 1 to 20 Hz. At frequencies above 20 Hz, with 5 ms pulse widths, some pulses failed to elicit an action potential and the higher the frequency the greater the failure rate; failure was often complete at 50 Hz, though a few cells fired at up to 120 Hz.

Of the 26 cells held long enough to examine the effect of focal stimulation of the associated fibre tracts, excitatory postsynaptic potentials (e.p.s.ps) were recorded in 12. Failure to detect e.p.s.ps in the other 14 cells does not necessarily imply that these cells lacked a synaptic input; in only one cell of the 26 were all accessible fibre tracts stimulated (see below) and in cells where no e.p.s.ps were detected an appropriate fibre tract may not have been stimulated.

Synaptic potentials were elicited at 1 Hz in most experiments and after the first few stimuli, when some run-down in amplitude was observed, the synaptic potentials remained approximately equal in amplitude. The synaptic potential elicited in response to a single focal stimulus was often followed by further spontaneous synaptic potentials (Figure 7c); since the time interval between these e.p.s.ps was not constant it would seem unlikely that processes of other cells activated by the same stimulus were making synaptic contact with the impaled cell. Perhaps the evoked release of transmitter facilitates 'spontaneous' release, as occurs in guinea-pig superior cervical ganglia over the initial 30 s stimulation period (McLachlan, 1975). Spontaneous miniature synaptic potentials (0.5 mV) were observed in a few preparations at a time when the preparation had not been stimulated but this was not a common observation.

If the synaptic potential reached threshold, an action potential was elicited (Figure 7d). Synaptic potentials were reversibly abolished by Tc (0.13 mM, 5 preparations) by C_6 (1.3 mM, 5 preparations) and

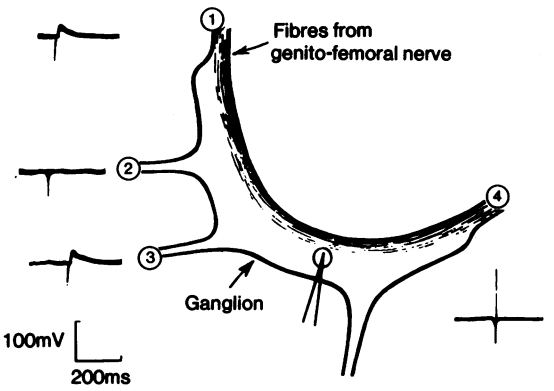

Figure 9 Drawing of a ganglion with accessory nerve fibres. Following impalement of a single cell in this ganglion (inset), focal stimulation of centrifugal fibres from the genito-femoral nerve at positions 1 and 3 produced synaptic potentials. Stimulation at position 2 gave no response and at position 4 an action potential which was not preceded by a synaptic potential and was presumed to be an antidromic spike. The absence of any synaptic step at position 4 was confirmed visually in the parent oscilloscope; the records were taken when the camera speed was inadvertently slowed. Traces retouched.

by DHBE (0.1 mM, 1 preparation) after 5 to 15 min exposure (Figure 7 e-g). In a few cells an action potential produced by stimulation of a nerve fibre tract was not preceded by a synaptic step and was unaffected by Tc or by C_6; on the basis of this and other accepted criteria (Blackman, Ginsborg & Ray, 1963), we take this action potential to have resulted from antidromic invasion of the cell body.

In one cell, all four accessible fibre tracts were stimulated with the focal stimulation electrode (Figure 9). Stimulation of the centrifugal fibre tract from the genito-femoral nerve (no. 1) elicited an e.p.s.p. Stimulation of an adjacent smaller tract (no. 2) produced no response and stimulation of the other small tract (no. 3) produced an e.p.s.p. Neither of these two smaller tracts was traced to its origin before the preparation was dissected free. Stimulation of the other accessible tract (no. 4), which ran in the direction of the muscle, produced a spike which was not preceded by a synaptic step and which was considered to be an antidromic spike. In this one preparation an explanation of these results would appear to be that some centrifugal fibres make synaptic contact with the ganglion cell impaled and that the cell axon lies in the tract which runs towards the muscle mass (no. 4), i.e. the ganglion cell may lie on one of the extrinsic nerve pathways to the muscle. Clearly the possibility that the cell also lies on a pathway to or from some neighbouring structure or indeed that it forms part of a local reflex arc similar to the reflex arcs found in the large intestine (Crowcroft, Holman & Szurszewski, 1971) and in the bladder (De Groat & Theobald, 1976) must also be considered.

Discussion

The extrinsic nerves to the rat anococcygeus ramify on the lateral surface to form a nerve network near the anal end of the muscle. On microscopic examination, cells resembling ganglion cells were found at junctions of this network and on occasion within the muscle mass itself. Preparations of the muscle dissected to remove the extrinsic nerves may therefore retain a small variable number of ganglion cells within the muscle mass in addition to postganglionic neurones. Field stimulation of such preparations may therefore be susceptible to ganglion blocking agents. The effectiveness of ganglion blocking agents in such preparations is likely to be very slight compared with preparations containing intact extrinsic nerves.

The results from experiments with Tc and DHBE are clearly the most important in trying to determine the functional relationship between the ganglion cells and the anococcygeus muscle. Each drug consistently reduced and, on occasions, abolished the contractions of the muscle elicited by extrinsic nerve stimulation. Although Tc initially depressed the response to added NA, due presumably to a non specific depressant effect on the muscle membrane, recovery occurred in the continued presence of Tc. This suggests that the inhibitory effect on the response elicited by nerve stimulation was not due to a non-specific effect on the smooth muscle membrane. These results indicated that at least part of the excitatory pathway to the muscle was interrupted by a synapse which may be in one of the small ganglia lying close to the muscle. However, since the response of most preparations to excitatory nerve stimulation could not be inhibited completely, part of the excitatory pathway may not pass through the ganglia studied. The results from the electrical experiments accord with this suggestion. Although it is impossible to say whether a particular ganglion cell impaled innervated the muscle, both Tc and DHBE, in similar doses to those used to inhibit motor nerve responses, reduced synaptic potentials elicited by stimulation of associated nerve fibre tracts.

C_6 showed both excitatory and inhibitory effects on the anococcygeus. The enhancement of the muscle response to motor nerve stimulation and to field stimulation was antagonized by phentolamine and so could have been mediated partly by an increased liberation of NA. This would also explain the reversal of the block produced by phentolamine. On the other hand C_6 also enhanced the motor response to added

NA even in 6-OHDA-treated preparations in which the adrenergic nerves are destroyed. This suggests an additional direct action on the smooth muscle membrane. Tetraethylammonium also augments the response of the anococcygeus to field stimulation by a presynaptic increase in the liberation of noradrenaline and this effect is sensitive to phentolamine. Tetraethylammonium additionally has a direct action on smooth muscle which is resistant to phentolamine (Gillespie & Tilmisany, 1976). It has been suggested that tetraethylammonium abolishes the rectifying properties of the membrane by reducing the normally high potassium permeability (Ito, Kuriyama & Sakamoto, 1970; Kirkpatrick, 1975). C_6 may have the same action.

Following the enhancement, C_6 inhibited the response to extrinsic nerve stimulation but *not* to field stimulation in the rat anococcygeus. This second action of the drug is probably at ganglia lying along the excitatory pathway; evidence from the intracellular recordings are consistent with this view. C_6 also reversibly abolished synaptic potentials after 5 to 15 min exposure and in this respect C_6 behaved like Tc. The action of drugs helped little in uncovering the organization of the inhibitory pathway. Inhibitory fibres are present in each of the extrinsic nerves and both Tc and C_6 often potentiated the inhibitory response of the muscle.

Intracellular electrical recordings, made while stimulating associated fibre tracts, confirmed that a synaptic input to at least some of the ganglionic neurones existed. When a synaptic potential had reached threshold, an action potential was elicited indicating that transmission occurred through these small ganglia. Spontaneous e.p.s.ps were rare. The ability of Tc, DHBE and C_6 to reduce the synaptic potentials indicated that synaptic transmission in these ganglia was cholinergic. No excitatory response was recorded electrically following C_6. This observation was consistent with the earlier suggestion that the site of the enhancement of the motor response in the muscle by C_6 lay postganglionically.

There were no distinguishing electrical features of the ganglion cells revealed by the present experiments. The input resistance and membrane potential of the neurones were comparable to those of several other mammalian ganglion cells given the limitations placed upon the accuracy of results employing such high resistance electrodes and a bridge circuit for the simultaneous passage of current and recording of resistance. In particular, their electrical characteristics resembled those of the guinea-pig pelvic ganglia previously described (Holman *et al.*, 1971). In both ganglia, spontaneous excitatory junction potentials were not recorded. Interestingly, we failed to find evidence of inhibitory potentials (i.p.s.ps), in spite of the presence of SIF cells.

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