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TRANSMISSION FROM PREGANGLIONIC FIBRES IN THE HYPOGASTRIC NERVE TO PERIPHERAL GANGLIA OF MALE GUINEA-PIGS

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SUMMARY

1. Intracellular records were obtained from ganglion cells of the pelvic plexus of male guinea-pigs.

2. The input resistance of cells which responded to intracellular stimulation varied from 40 to 150 MΩ. Slope resistance decreased when the membrane was hyperpolarized. Time constants varied from 5 to 200 msec. Resting membrane potentials ranged from 40 to 70 mV.

3. Action potentials in response to direct stimulation were followed by a prolonged phase of after-hyperpolarization.

4. A second type of cell was also impaled which did not respond to electrical stimulation. These cells had resting membrane potentials in the range 60–70 mV, input resistances of less than 20 MΩ and time constants of less than 3 msec.

5. In most ganglion cells, stimulation of the hypogastric nerve evoked action potentials which were often followed by a secondary phase of depolarization indicating continuing transmitter action.

6. Orthodromic responses were generally 'all-or-nothing' and could not be graded with changes in stimulus strength. The latency of orthodromic responses indicated that ganglion cells were innervated by both B and C fibres in the hypogastric nerve.

7. Orthodromic responses were blocked by tubocurarine, 5×10^{-5} g/ml., and dihydro-β-erythroidine, 10^{-5} g/ml.

8. Spontaneous, excitatory post-synaptic potentials of up to 4·8 mV in amplitude were observed. The frequency of their discharge was greatly increased by repetitive stimulation of the hypogastric nerve.

9. The ultrastructure of the pelvic ganglia was studied by electron-microscopy. Two types of ganglion cell process were observed, fine (0·1 μ)

branching tufts thrown up from the soma within the surrounding capsule and longer, thicker (1 μ) extracapsular processes. Synapses were found to occur most frequently between the varicose terminal segments of preganglionic axons and the small intracapsular processes.

10. Similarities between the properties of the pelvic ganglia innervated by the hypogastric nerve and those of the parasympathetic division of the autonomic nervous system are discussed.

INTRODUCTION

Our present understanding of the transmission of excitation in mammalian autonomic ganglia is largely derived from studies on the electrical activity of intact ganglia recorded with extracellular electrodes. On the other hand much information at the cellular level, has been gained from micro-electrode studies on the autonomic ganglia of amphibia and ganglion cells of invertebrates (see Tauc, 1967). The only detailed intracellular work on mammalian ganglia is that of Eccles (1955, 1963), who studied the superior cervical ganglion of rabbits.

Mammalian ganglia are surrounded by a tough, protective sheath of collagen which is not easily penetrated by glass micro-electrodes (Eccles, 1955). We decided to investigate the pelvic ganglia of male guinea-pigs since many of these are quite small and we hoped that their connective tissue investment might present a less impenetrable barrier than that of the sympathetic chain. Furthermore, it was known that pelvic ganglia innervated by the hypogastric nerve survive well *in vitro* (Hŭkovic, 1961).

The noradrenergic ganglion cells of the pelvic plexus are of interest, in that they differ in a number of ways from the generally accepted model of a mammalian sympathetic ganglion cell, as exemplified by those of the superior cervical ganglion. The pelvic ganglia have short axons and are situated close to their target organs (Sjöstrand, 1965). They are resistant to the action of anti-nerve growth factor (Hamberger, Levi-Montalcini, Norberg & Sjöquist, 1965) and to the action of certain specific sympathetic ganglion stimulants (Bentley, 1968). Their terminals are relatively resistant to the catecholamine depleting action of reserpine (Sjöstrand & Swedin, 1968). In the guinea-pig, some, but not all pelvic ganglia are supplied with noradrenergic nerve terminals and groups of chromaffin cells are commonly found within them (Sjöstrand, 1965).

This paper describes the ultrastructure of pelvic ganglion cells, their electrical properties and the main features of their response to stimulation of preganglionic fibres in the hypogastric nerve. Preliminary accounts of some of this work have been published (Blackman & Holman, 1967; Devine, 1967).

METHODS

Morphology. Pelvic ganglia were dissected from young male guinea-pigs as for the electrophysiological experiments described below and fixed in a 2% glutaraldehyde-2% formaldehyde mixture (Karnovsky, 1965) buffered to pH 7·4 with sodium cacodylate. They were then washed in fresh buffer and post-fixed in 1% osmium tetroxide. The tissues were dehydrated in ethanol and embedded in Epon 812. Some ganglia were used for electrophysiological experiments before fixation. Sections were cut with a Porter Blum ultramicrotome, stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined in a Hitachi HU 11A electron microscope. Thicker sections were cut and stained for examination with the light microscope.

Electrophysiology. The hypogastric nerve and its peripheral branches were dissected as rapidly as possible from male guinea-pigs which had been stunned and bled. The preparation was pinned out in an isolated organ bath (2 ml. capacity) which was illuminated from below. The sheets of connective tissue overlying the ganglia were removed until individual cells within the ganglion were visible at a magnification of $\times 25$ or $\times 50$.

The bath was maintained at 33–35° C and perfused at a rate of 2–3 ml./min with a physiological salt solution of the following composition (mM): NaCl, 120; KCl, 5·0; $CaCl_2$, 2·5; $MgSO_4$, 1·0; $NaHCO_3$, 25; NaH_2PO_4, 1·0; glucose, 11. Solutions were equilibrated with 95% O_2 and 5% CO_2.

The hypogastric nerve was stimulated with a suction electrode (Blackman, Ginsborg & Ray, 1963) or by two rings of Pt wire embedded in Araldite (epoxy resin, CIBA Ltd) (Burnstock & Holman, 1961). A Grass S4 stimulator was used throughout. Values of stimulus strength given in the text refer to the setting of the stimulator and not to the absolute value of the voltage between the stimulating electrodes. Unless otherwise stated, the pulse duration was 0·5 msec. Frequencies of stimulation were less than 1/sec.

Membrane potentials were recorded by conventional glass micro-electrodes filled with 3 M-KCl. A unity gain preamplifier (Picometric) was used to couple the micro-electrode to the recording apparatus (Tektronix 502 or 565 cathode ray oscilloscope). A Wheatstone bridge circuit was used to pass current through the electrode used for recording membrane potential (Martin & Pilar, 1963; Hashimoto, Holman & Tille, 1966). The fixed resistance of the bridge was 1000 MΩ.

Blockage of the recording electrode was a continual hazard throughout these experiments. From time to time, d.c. calibrating steps of 10 mV were led into the bath via the indifferent electrode (Martin & Pilar, 1963) but we could not exclude the possibility that the values of membrane potential obtained in many of the early experiments were underestimated. No attempt was made to correct for the input time constant of the recording system, and the amplitude of many of the action potentials was undoubtedly attenuated. More recent attempts to find the absolute values for peak amplitude, rate of rise, etc., will be described in a later paper.

RESULTS

Morphology

In low power electron micrographs, each ganglion was seen to contain a variable but rather small number of ganglion cells; in one ganglion the number was estimated to be about 200. Chromaffin cells were seen clustered around the axon bundles entering or leaving the ganglion. Under higher magnification (Plate 2*B*) long, branching processes of chromaffin cells with granules approximately 1500 Å in diameter were seen to ramify

extensively throughout the ganglion. Ganglion cell bodies, about 20–30 μ in diameter, were tightly packed together in groups with larger tissue spaces between them. Within these larger spaces there were fibre tracts containing both myelinated and unmyelinated axons (0·5–3·0 μ in diameter). Each ganglion cell, staining with moderate intensity, contained endoplasmic reticulum and ribosomes.

The narrower spaces between individual ganglion cells within a group were occupied by the capsule cells surrounding each cell body and by small bundles of nerve fibres ensheathed by Schwann cells. Schwann and capsule cells could be distinguished from ganglion cells by their less densely stained cytoplasm and their characteristic thin filaments (Plate 1). The nerve fibres could usually be classified as either preganglionic axons or ganglion cell processes as described previously by Elfvin (1963*a*, *b*). The cytoplasm of the ganglion cell processes resembled that of the cell bodies. Myelinated or unmyelinated preganglionic axons were identified by their much less densely stained cytoplasm and the vesicles present within them. Two types of vesicles were observed; small, agranular vesicles approximately 500 Å in diameter and larger vesicles, almost filled by a granule of variable density, approximately 900 Å in diameter.

The terminal segments of preganglionic fibres resembled those described by Elfvin (1963*a*, *b*). The preganglionic axons which made synaptic contacts with ganglion cells on their processes were varicose; narrow segments, containing neurotubules but no synaptic vesicles, alternated with wider segments which were often tightly packed with vesicles (Plate 2*A*).

Clusters of fine, 0·1–0·3 μ diameter, ganglion cell processes were seen within the capsule (Plate 2*A*). These short, interweaving structures only rarely contained cellular components. In a limited number of serial sections they were shown to have the form of branching tufts thrown up from the surface of the ganglion cell.

Synapses occurred more commonly between the varicose regions of preganglionic axons and the fine ganglion cell processes within the capsule, although they were also found on the extracapsular processes and on the cell body itself. At synapses, the pre- and post-synaptic membranes were thickened and spaced 100–150 Å apart (Plate 2*A*) for distances up to 5000 Å.

Electrophysiology

Initial observations. During the early stages of this work it was apparent that at least two different types of cell could be impaled as the microelectrode entered the ganglion. One group of cells did not respond to orthodromic or to intracellular stimulation. Their resting potentials ranged from 60 to 70 mV and the maximum steady value of their potential was usually established immediately after penetration. Rectangular

current pulses caused changes in membrane potential of rapid time course (10–90 % rise time, less than 3 msec) often similar to those observed when the electrode was extracellular and the bridge unbalanced. Their input resistance was generally less than 20 msec. Since these cells did not respond to stimulation, we looked for other evidence which might indicate whether or not the microelectrode was actually inside a cell. When the extracellular K^+ concentration was increased by adding a drop of 2 M-KCl to the perfusing solution, the potential fell by up to 40 mV to return later to its original level as the high K^+ solution was washed out of the bath.

We have tentatively concluded that these observations indicated the impalement of a capsule cell or possibly a chromaffin cell.

The second type of cell responded to intracellular stimulation with an action potential of the spike form. In many instances, especially during initial experiments, impalement of these cells was associated with a less abrupt change in potential than that characteristic of the impalement of inexcitable cells. A discharge of spikes was sometimes observed at frequencies of up to 30/sec. As such impalements stabilized, the frequency of the discharge gradually decreased until, after times ranging from a few seconds to several minutes, the discharge ceased and a resting membrane potential of 50–70 mV was established. The resting potential of some cells continued to increase for a further 5 min or so, as described previously by Blackman *et al.* (1963). As we gained experience with this preparation, ganglion cells were impaled without evidence of an initial injury discharge. Nevertheless, the recorded resting potentials of all electrically excitable cells was rather low, ranging from 40 to 70 mV. We cannot exclude the possibility that the micro-electrode caused a variable degree of injury.

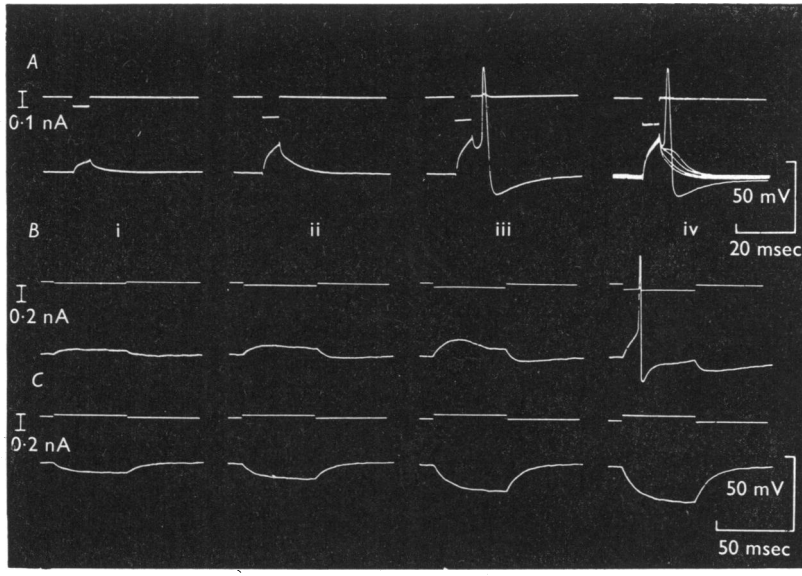
About 60 % of the cells responding to intracellular stimulation also responded to stimulation of the hypogastric nerve. Those not responding may have been innervated by preganglionic fibres of sacral origin. Alternatively, some of the preganglionic fibres in the hypogastric nerve may have been damaged during dissection. Since the diameter of nerve fibres observed within the ganglion was small (up to 3 μ), it was assumed that these responses were recorded from the soma of the ganglion cells.

A third group of cells, which did not respond to direct or to orthodromic stimulation, had high input resistance (greater than 100 MΩ) and their voltage responses to current pulses had slow rise times. It was possible that these observations were the result of blocked electrodes and such results were discarded.

The results given in the remainder of this paper refer to cells which responded to electrical stimulation or to cells which responded to orthodromic stimulation but were lost before there was time to check their response to intracellular stimulation.

Electrical properties of ganglion cells

Effect of depolarizing currents. Text-figure 1 *A* and *B*, shows the effect of depolarizing currents of increasing intensity on the membrane potential recorded from two typical ganglion cells. In *A*, the current pulse was of 5·5 msec duration and the local potential, from which the action potential developed, outlasted the duration of current flow (see record *A*, iv). Text figure 1 *B* shows the sequence of events leading to excitation by a current



Text-fig. 1. Effect of current pulses (upper tracing in each record), applied through an intracellular electrode, on the membrane potential (lower tracings) of two ganglion cells. The records of row *A* show the effect of increasing currents of relatively brief duration. Record *A*, iv shows superimposed traces for a series of current pulses of threshold intensity. The records of rows *B* and *C* were from a different ganglion cell and show effects of depolarizing (row *B*) and hyperpolarizing (row *C*) currents.

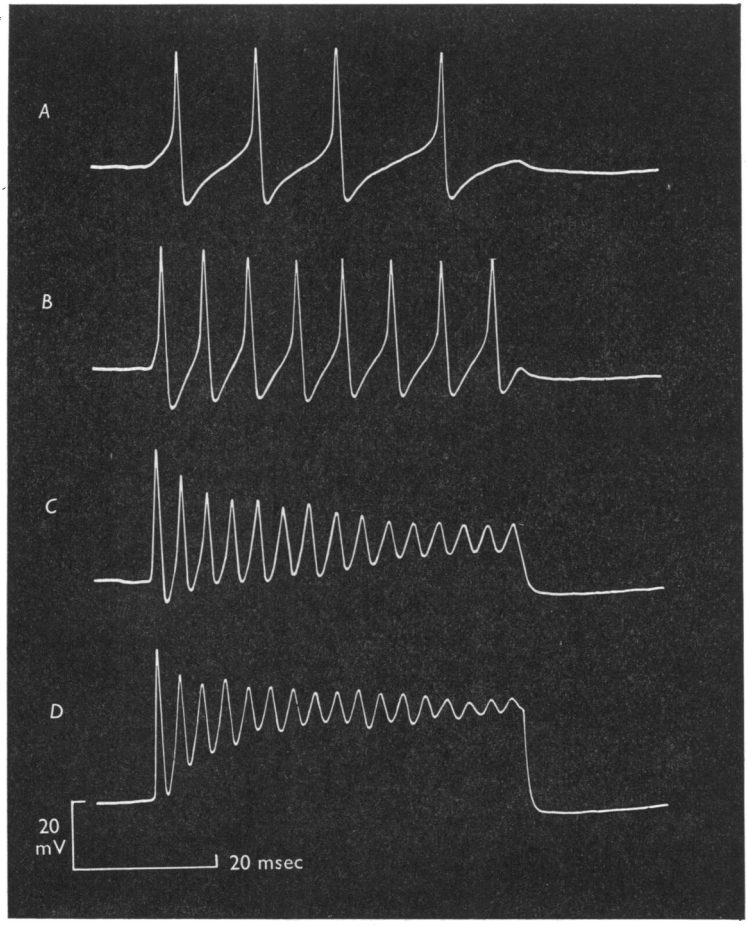
pulse of longer duration (45 msec). For comparison, the records of Text-fig. 1 *C* show the effects of hyperpolarizing currents on the same cell as that of Text-fig. 1 *B*.

Currents of suprathreshold intensity caused repetitive firing of action potentials, as shown in Text-fig. 2. The interval between successive action potentials decreased with currents of increasing intensity. When the interval between them was less than 6 msec, successive action potentials deteriorated into a damped oscillation, as in Text-fig. 2 *C* and *D*.

Provided the depolarizing current was small or 'turned off' before or

during an action potential, repolarization always led to a phase of after-hyperpolarization. The time course of this potential is illustrated in Text-fig. 3.

There was considerable variation in the values of the parameters describing these responses to intracellular stimulation. Some representa-

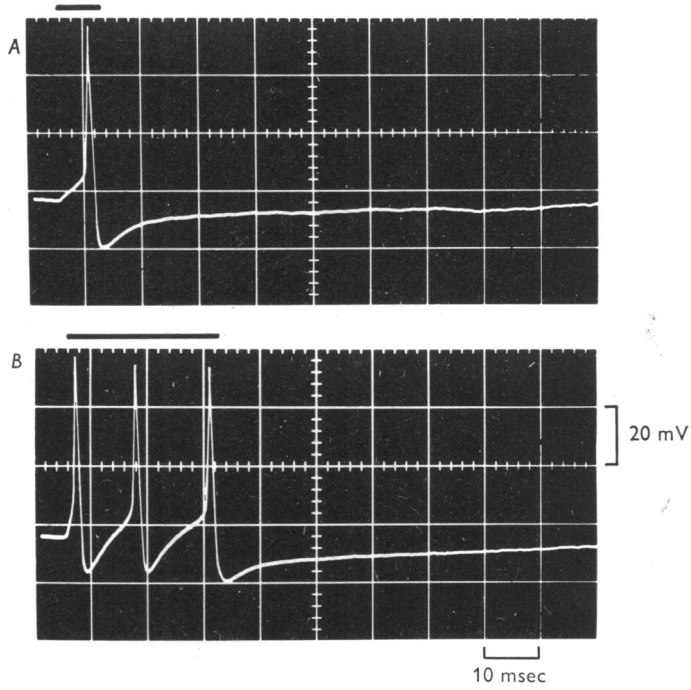


Text-fig. 2. Changes in membrane potential in response to currents of increasing intensity: *A*, 0·1; *B*, 0·25; *C*, 0·8; and *D*, 1·1 nA.

tive values are given in Table 1. In general, the amplitude of the repolarization phase, from the peak of the action potential to the maximum level of repolarization, was fairly constant, varying from 85 to 90 mV. Although the true value of the resting membrane potential was often uncertain it was apparent that the cells with large after-hyperpolarizations had the lower resting potentials. Furthermore, cells with low resting potentials

always had low thresholds for excitation, and vice versa. There was a highly significant negative correlation between threshold depolarization and the maximum level of hyperpolarization (correlation coefficient = −0·59).

Effects of hyperpolarizing currents. In approximately two thirds of cells, small hyperpolarizing currents caused changes in membrane potential



Text-fig. 3. Records showing the time course of the after-hyperpolarization following a single action potential (*A*) and three action potentials (*B*) in response to intracellular stimulation. The duration of the current pulse is indicated by the horizontal bar above each trace. Current intensity, 0·1 nA (*A*) and 0·35 nA (*B*).

TABLE 1. Characteristics of responses to intracellular current flow

Cell no.	Threshold current (10^{-10}A)	Threshold depolarization (mV)	Amplitude of action potential (mV)	After-hyperpolarization (mV)	R_{input} (MΩ)	τ (msec)
49	1·5	20	78	6·5	100	7·0
59	0·8	17	67	18·0	150	13·0
65	1·3	13	72	18·0	65	7·0
68	0·8	12·5	70	19·5	125	8·5
69	3·0	18·0	75	15·0	50	6·0
70	1·1	16·0	70	16·0	145	18·0
24	0·8	11·5	70	11·0	120	6·5
113	0·5	16·0	76	13·0	240	11·0

whose time course could be closely approximated by a single exponential function (see Text-fig. 1*C*). The remaining third showed 'overshoots' and 'undershoots' at the onset and cessation of the current pulse, similar to those described for cat motoneurones by Ito & Oshima (1965). In cells with low resting potentials, 'overshoots' and 'undershoots' in the depolarizing direction often gave way to a local response or to excitation.

The time constants given in Table 1 were calculated for changes in membrane potential of less than 10 mV, recorded from cells in which changes in membrane potential were single exponential functions. Values for thirty such cells ranged from 6 to 10 msec. Cells with relatively long time constants also had high values of input resistance.

Input resistance was estimated from graphs of the relation between current intensity and the steady level of hyperpolarization measured 20–100 msec after the onset of current. This was usually linear for hyperpolarizations of less than 10 mV and a straight line could be drawn through these values and the origin. Cells responding with marked 'overshoots' and 'undershoots' were omitted from this analysis.

Text-figure 4*A* is a histogram of the values of input resistance measured in this way for thirty-seven cells. The estimated values of input resistance at the extremes of the range are probably unreliable, since, depending on the value, any blockage of the electrode would lead to either an underestimate of the change in membrane potential or to lack of bridge balance. Our results suggest that input resistances probably vary from 40 to 150 MΩ and that values are distributed unimodally within this range.

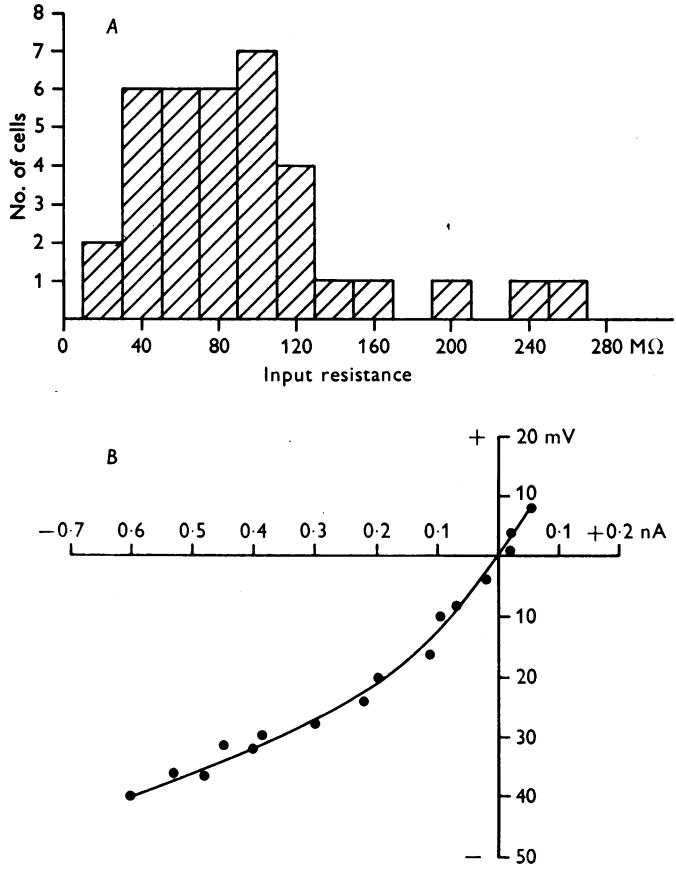
The slope of the relation between current and change in membrane potential was not constant and all ganglion cells showed some degree of anomalous rectification for currents causing hyperpolarizations of more than 10 mV. This is illustrated in the graph of Text-fig. 4*B*.

Responses to orthodromic stimulation

General characteristics. The sequence of changes in membrane potential in response to orthodromic stimulation resembled those described previously for frog sympathetic ganglia (Nishi & Koketsu, 1960; Blackman *et al.* 1963). Text-figure 5 illustrates the various types of response observed in this study.

Text-figure 5*A* is an example of prolonged and intense excitatory transmitter action which led to the firing of two action potentials. Eight out of a total of eighty cells behaved in this way. In the cell shown in Text-fig. 5*A* the amplitude of both action potentials (73 and 66 mV respectively) was lower than that of the action potential in response to intracellular stimulation (80 mV). The interval between the two action potentials of Text-fig. 5*A* was 9 msec. It is possible that the large depression of the

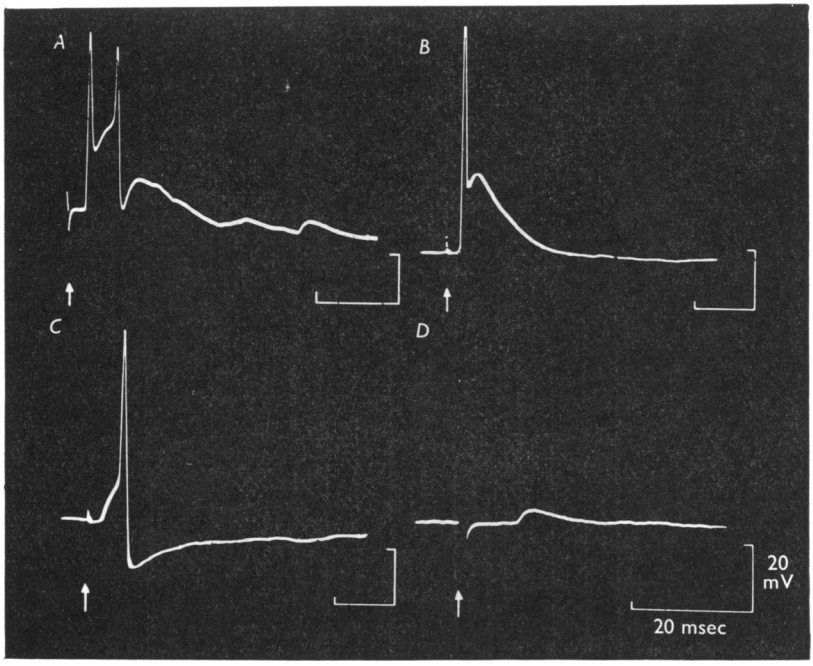
second action potential could have been due to the relative refractory period of the first. Alternatively, maximum intensity of transmitter action may not have developed at a time when the first action potential had reached its peak, only 1 msec after the onset of depolarization. In five of the cells giving two action potentials in response to a single orthodromic stimulus, the first action potential was depressed more markedly



Text-fig. 4. *A*. Histogram of values of input resistance calculated for thirty-seven cells. *B*. Relation between current (nA) and membrane potential (mV) for a typical cell.

than the second. In four of these, the amplitude of the second action potential was almost equal to that of the direct response, indicating that the intensity of transmitter action was probably decreasing at that time (10–15 msec after onset of depolarization). In every case, the threshold depolarization for initiation of a second action potential was high (30–40 mV compared with the maximum of 25 mV for initiation of a single action potential by less intense synaptic action).

Text-figure 5*B* is an example of the commonest type of orthodromic response. In such cases, the amplitude of the action potential was always depressed compared with that in response to direct stimulation. Depolarization followed the action potential but did not reach threshold for the firing of a second action potential. In other cells of this type the 'post-spike' depolarization varied in amplitude from 35 to 1 or 2 mV. In some cases, this depolarization was evident as a 'hump' on the hyperpolarization which followed the action potential (Text-fig. 6*A*).



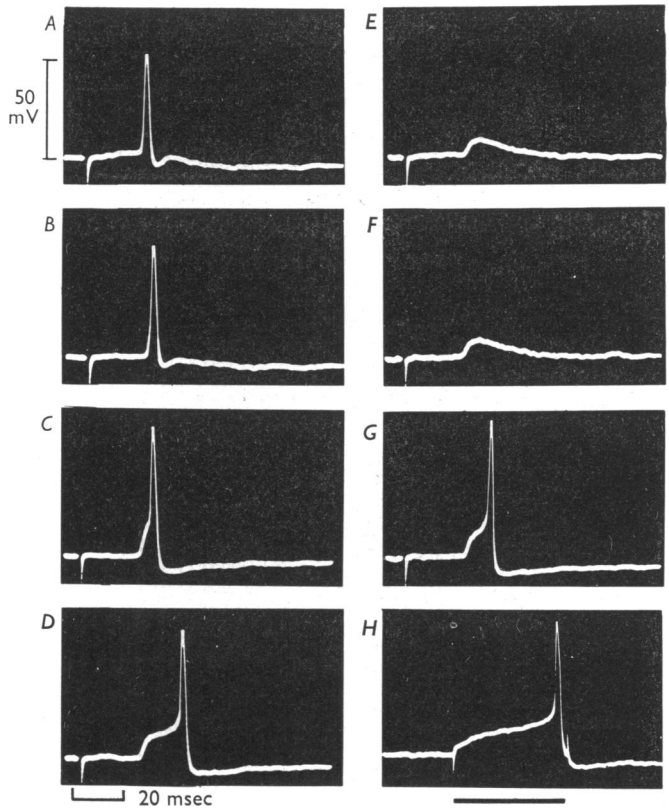
Text-fig. 5. Responses to stimulation of the hypogastric nerve recorded from four different cells. Stimulus artifacts are indicated by arrows. See text for explanation.

Text-figure 5*C* shows an example of less intense transmitter action. A 'synaptic step' is evident in the rising phase of the action potential which was followed by a phase of after-hyperpolarization whose amplitude and time course was similar to that of the response to direct stimulation (compare Text-fig. 5*C* with Text-fig. 3).

Eight cells responded to orthodromic stimulation with excitatory post-synaptic potentials (EPSPs) like that shown in Text-fig. 5*D*. In some of these cells, successive EPSPs fluctuated in amplitude, occasionally reaching threshold for firing of an action potential (Text-fig. 8). In other cases, facilitation of EPSPs by repetitive stimulation brought them up to

threshold for excitation. The duration of subthreshold EPSPs varied from 17 to 35 msec.

Text-figure 6 illustrates the effects of progressively diminishing transmitter action, as a result of partial blockade by tubocurarine (5×10^{-5} g/ml.). The control orthodromic response (Text-fig. 6*A*) consisted of an action potential of 55 mV amplitude. As transmission was gradually

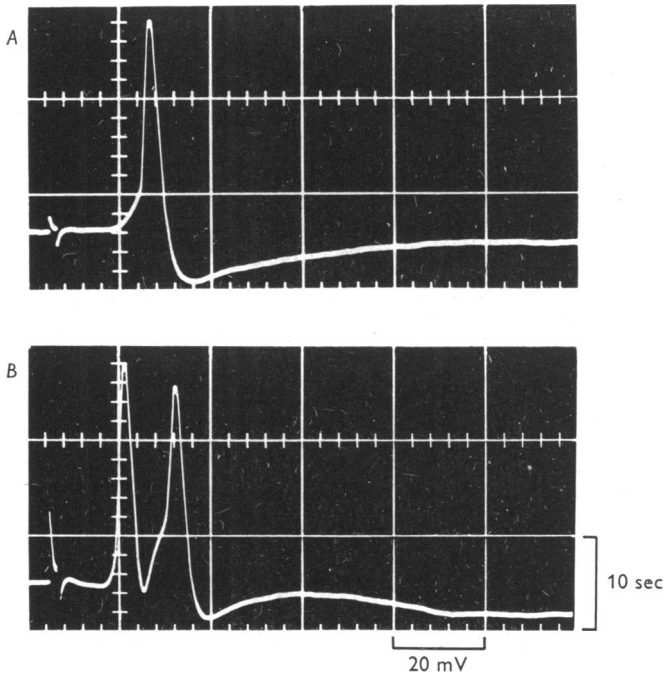


Text-fig. 6. Effect of tubocurarine, 5×10^{-5} g/ml. *A*, control response to hypogastric nerve stimulation recorded in normal solution; *B*, *C*, *D*, *E*, *F* and *G* recorded approximately 10, 30, 35, 40, 50 and 60 min respectively after the addition of the drug; *H*, response to intracellular stimulation (duration of current pulse indicated by horizontal bar).

depressed, the amplitude of the action potential increased until, in Text-fig. 6*G*, it was similar to that in response to intracellular stimulation (Text-fig. 6*H*). After 40 min exposure to tubocurarine, the majority of responses to orthodromic stimulation were pure EPSPs such as those illustrated in Text-fig. 6*E* and *F*. However, successive responses fluctuated

(see below) and occasionally an EPSP reached threshold for initiation of an action potential (Text-fig. 6*G*).

Absence of 'grading' of response. The responses of pelvic ganglion cells to stimulation of the hypogastric nerve were predominantly 'all-or-nothing' in nature. In all but three of more than thirty cells which were held for long enough to test the effect of varying the strength of stimulation from threshold up to the maximum output of the stimulator, there was no



Text-fig. 7. Change in response to orthodromic stimulation as a result of increased strength of stimulation. See text for explanation.

change in latency or in the nature of the response. Thus, if the response to threshold stimulation was a pure EPSP, increasing the strength of stimulating up to 10 or more times threshold failed to cause an increase in amplitude.

One of the cells which responded to an increase in stimulus strength with a change in the nature of its response is shown in Text-fig. 7. A stimulus of 3·8 V elicited an action potential by relatively weak transmitter action (Fig. 7*A*). An increase in stimulus strength to 14 V caused a decrease in the latency of the response and an increase in transmitter action so that two action potentials were initiated (Fig. 7*A*). In all other cells whose response varied with stimulus strength, an increase in strength

caused additional depolarization but only after a long latency. In most cases the minimum stimulus strength for excitation of the cell varied from 2 to 4 V (0·5 msec duration). Stimuli of twice this minimum value were usually adequate to evoke maximal orthodromic responses.

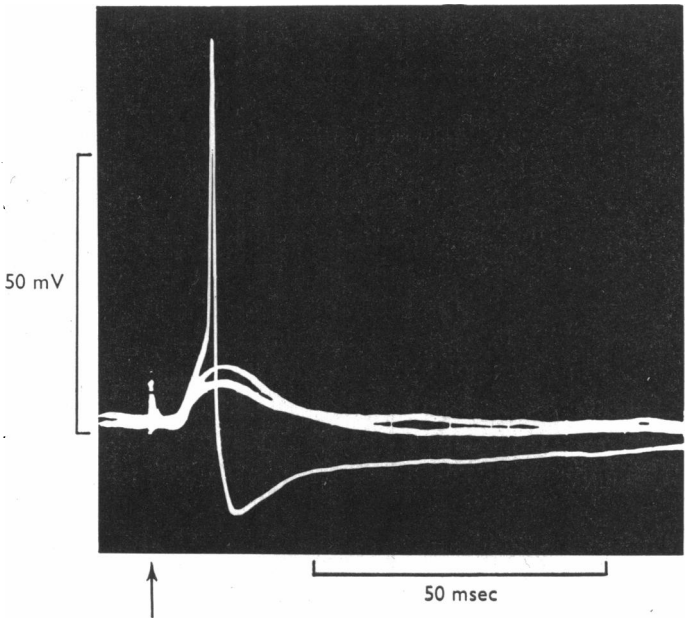
Latency. The latency of orthodromic responses ranged from 5 to 35 msec. This variation was due, in part, to differences in the length of the preganglionic nerve between the stimulating electrodes and the ganglion under study. However, in eight preparations, cells with widely differing latencies were obtained from within the same ganglion. For example, in one ganglion, five cells had latencies of 5·0, 6·3, 15, 16 and 16 msec respectively. This ganglion was situated approximately 10 mm from the stimulating electrode but the actual conduction distance for the preganglionic fibres must have been greater than this. The shortest latency responses were probably due to excitation of fibres with a conduction velocity greater than 2 m/sec, and the longest latency responses to fibres conducting at about 0·6 m/sec. Similar estimates for other preparations indicated that the pelvic ganglion cells of the guinea-pig are innervated by hypogastric nerve fibres with conduction velocities ranging from 5 to less than 0·4 m/sec. However, it must be emphasized that no attempts were made specifically to determine conduction velocities, and furthermore, that the synaptic delay within the ganglion is unknown.

According to Ferry (1967) there are three components of the B fibre elevation of the compound action potential of the guinea-pig hypogastric nerve. The fastest has conduction velocities ranging from 7 to 10 m/sec; the major elevation, velocities from 4 to 7 m/sec, and the third and smallest group, velocities of 1·5–4 m/sec. The C fibre elevation is conducted at 0·5 m/sec. It is difficult to explain the longest latencies observed in the present experiments in terms of the conduction velocities of preganglionic B fibres. It seems likely that some of the C fibres in the hypogastric nerve must be preganglionic axons.

For any preparation, the threshold for excitation of a short latency orthodromic response was lower than the threshold for a long latency response. The only exception to this rule was the cell shown in Text-fig. 7.

Fluctuation of responses to orthodromic stimulation. All cells showed evidence of fluctuation in the intensity of the transmitter action during repeated stimulation at frequencies of up to 1/sec. This fluctuation was independent of stimulus strength and was just as evident for stimuli a little above threshold as for stimuli of more than 10 times threshold intensity. In many cells, whose orthodromic response was similar in form to that of Fig. 5*B*, the depolarization following the action potential varied in amplitude and time course from one response to the next. In other cells, the amplitude of the action potential also varied.

Text-figure 8 shows an extreme example of a fluctuating response. In this cell, successive stimuli at 0·5/sec, evoked sub-threshold EPSPs of variable amplitude or occasionally a supra-threshold EPSP and an action potential. Fluctuations in transmitter action were also observed in the presence of tubocurarine 5×10^{-5} g/ml., (see Text-fig. 6), and dihydro-β-erythroidine, 10^{-5} g/ml.



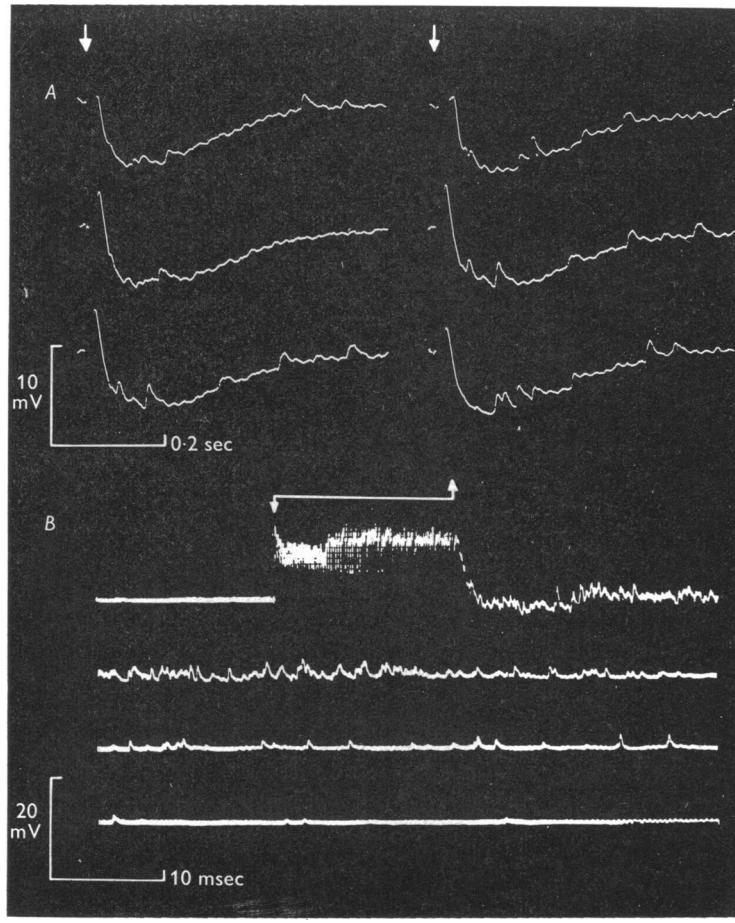
Text-fig. 8. Superimposed traces showing three successive responses to orthodromic stimuli of constant strength. Arrow indicates stimulus artifact.

Effect of changes in membrane potential on responses to orthodromic stimulation. Ganglion cells were hyperpolarized by current flow from an intracellular electrode following stimulation of the hypogastric nerve. In cells giving a response like that of Text-fig. 5*B*, the peak of the action potential remained at a constant level of membrane potential during hyperpolarization and thus the total amplitude of the EPSP-action potential complex increased, in parallel with the degree of hyperpolarization. A 'synaptic step' became apparent on the rising phase of the action potential and the peak of the action potential was delayed. In most of the cells tested, it was not possible to block the initial action potential evoked by orthodromic stimulation. This finding may be taken as further evidence for the intense excitatory transmitter action characteristic of the majority of units obtained from this preparation. In contrast, if a ganglion cell responded to orthodromic stimulation with two action potentials (e.g.

Text-fig. 5*A*), hyperpolarization by up to 10 mV was usually sufficient to block the second action potential.

In order to test the effect of changes in membrane potential on the EPSP, dihydro-β-erythroidine was used to produce partial block of transmission. It was found, however, that although it was possible to increase the amplitude of the EPSP by hyperpolarization, the data could not be used to determine the absolute value of the reversal potential for transmitter action for the following reasons:

(i) fluctuations in the amplitude of successive EPSPs,



Text-fig. 9. *A*, a series of records showing changes in membrane potential occurring during intervals of 0·5 sec after orthodromic stimulation (indicated by arrows). This cell was hyperpolarized following each evoked action potential (not recorded). Note the occurrence of small 'spontaneous' EPSPs as the membrane potential returns to normal. *B*, continuous record of the effect of a tetanus (10/sec for 16 sec, indicated by arrows) on the frequency of 'spontaneous' EPSPs.

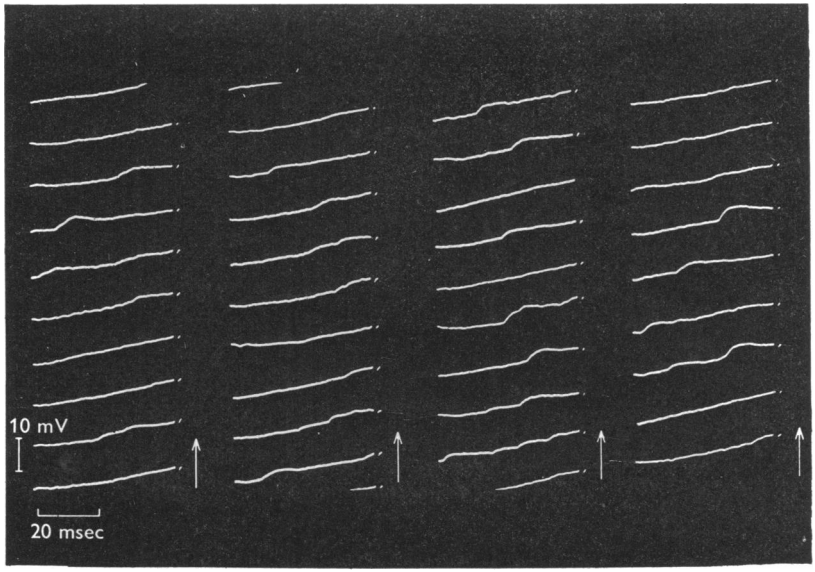
(ii) uncertainty of the value of the resting membrane potential,

(iii) the decrease in input resistance which occurred during hyperpolarization.

Present experiments are aimed at overcoming these difficulties.

Spontaneous EPSPs

Spontaneous EPSPs were rarely seen in the absence of orthodromic stimulation. In those cells where they could be recorded under resting conditions, their amplitude varied from 4·8 mV down to the noise level of the base line. However, their occurrence frequency was too low to



Text-fig. 10. Records of 'spontaneous' EPSPs photographed during 50 msec intervals immediately preceding stimuli to the hypogastric nerve at a frequency of 0·5/sec. Arrows indicate sequence in which traces were recorded.

permit analysis of their time course or the statistical distribution of the intervals between them. Orthodromic stimulation markedly increased the occurrence frequency of the spontaneous EPSPs, as shown in Text-fig. 9*A*. This effect was even more pronounced following high frequency repetitive stimulation (Text-fig. 9*B*).

The variation in amplitude and time course of the spontaneous EPSPs is shown in the records of Text-fig. 10. In this experiment the hypogastric nerve was stimulated at a rate of 0·5/sec and the trace photographed during the 50 msec preceding each stimulus. Data obtained from this and similar experiments indicated that the total rise time of the spontaneous EPSPs ranged from 3·5 to 7·0 msec, their half decay time from 5 to 10 msec, and their total duration from 15 to 30 msec.

DISCUSSION

These experiments suggest that there is a high safety factor for transmission of excitation from preganglionic fibres in the hypogastric nerve to many of the ganglion cells of the pelvic plexus of the male guinea-pig. However, it must be emphasized that the resting potential recorded in many cells was low, due probably to the effects of impalement. If the true value of the resting membrane potential *in vivo* is about 80 mV (Eccles, 1955) then it is possible that the intensity of transmitter action, such as that characteristic of the EPSP action potential complex of Text-fig. 5 *C*, would not normally have been sufficient to generate an action potential. On the other hand, the majority of cells encountered during these experiments responded to orthodromic stimulation in a manner which suggested far more intense transmitter action (e.g. Text-fig. 5 *A*, *B*). Moreover, hyperpolarization by current flow through the micro-electrode did not block excitation, although the membrane potential was increased to at least 80 mV.

The sharp threshold for an orthodromic response, and the absence of grading in the response of most cells to changes in stimulus strength, suggests that excitation of a ganglion cell results from the excitation of a single preganglionic fibre. If this can be confirmed it will be interesting to know if the same preganglionic fibre can excite more than one ganglion cell.

A contrasting picture has emerged from studies on ganglia of the thoracic sympathetic chain of the guinea-pig (Purves & Blackman, 1968). Both spatial and temporal summation have been demonstrated in these ganglia, which would therefore appear to be capable of integrative action. Preliminary electron microscopic studies on this preparation have indicated that the intracapsular ganglionic processes are far more numerous and extensive than those of the pelvic ganglia of the same species (C. E. Devine, unpublished work). Electron microscopic studies on the superior cervical ganglion (Elfvin, 1963*a*, *b*) have also demonstrated more numerous extracapsular processes than those of the pelvic ganglia. Again, the superior cervical ganglion has the capacity for integrative action (Eccles, 1944). Thus the morphology of autonomic ganglion cells may reflect their differing functions, whether as simple relays (e.g. the avian ciliary ganglion; Martin & Pilar, 1963), as a means of distr' ..ing the excitation of a single preganglionic fibre to many post-ganglionic fibres, or as a 'centre' for integration of the action of a number of different preganglionic fibres. Traditionally, the ganglia of the parasympathetic division of the autonomic nervous system are considered to function mainly as relays or distributing centres. The pelvic ganglia appear to belong to this category; they do not appear to be capable of integrative activity.

The peripheral location of the pelvic ganglia (Sjöstrand, 1965), their resistance to immunosympathectomy (Hamberger *et al.* 1965) and to the action of the specific sympathetic ganglion stimulant drug McN-A-343 (Bentley, 1968), also suggest a resemblance to the properties of ganglia of the parasympathetic division of the autonomic nervous system. The characteristic physiology and pharmacology of different autonomic ganglion cells may turn out to be associated with their position in relation to the neuraxis. It would seem that this factor is more significant in determining their properties than the nature of the transmitter which is released from their terminal axons.

As already noted, the majority of synapses on the pelvic ganglia appear to lie on the small tufts of intracapsular cell processes. The function of these processes is unknown. They may play a role in transmission analogous to that of the infoldings of the skeletal muscle membrane at the neuromuscular junction. Here the increased surface of the post-junctional membrane provides an extensive area which is occupied by post-junctional nicotinic receptors.

There seems no reason to doubt that the excitatory transmitter in these ganglia is acetylcholine since orthodromic responses were blocked both by tubocurarine and dihydro-β-erythroidine. It is of interest to consider what factors determine the duration of the post-synaptic action of acetylcholine. The duration of the EPSP in the cat superior cervical ganglion (Eccles, 1944) and in frog sympathetic ganglia (Blackman *et al.* 1963) is only slightly increased by anticholinesterase agents. It has therefore been suggested that the time course of the action of acetylcholine may be largely determined by diffusion away from its site of release (Emmelin & MacIntosh, 1956). Ogston (1955) calculated that the time course of the EPSP could be explained by such a process. Our results suggest that another factor may be important in determining the duration of transmitter action in response to a single orthodromic stimulus. In general, the duration of the evoked depolarization outlasted that of a spontaneous EPSP. It is possible that the probability for release of transmitter may remain at a supranormal level for some time after an axon terminal has been invaded by an action potential. Thus, part of the cause of prolonged transmitter action may be the continuing output of transmitter.

The electrical properties of the ganglion cell membrane were estimated by assuming that the cells were spherical, of variable diameter (20–30 μ), and that the contribution of the cell processes to the surface area of the soma was negligible. Values for the resistance of unit area of membrane ranged from 2000 to 10,000 Ω cm^2. The corresponding values for the capacitance of unit area of membrane were high (2–5 μF/cm^2). It seems preferable to accept that the membrane capacitance does not differ signi-

ficantly from that established for many other cell membranes (Katz, 1966) and that our calculations underestimate the surface area of the cell. However, it should be noted that Nishi, Soeda & Koketsu (1965) obtained values of 13–63 μF/cm^2 for toad sympathetic ganglion cells.

Throughout this work we have been impressed by the relative ease of impaling what we have tentatively concluded to be capsule cells. If these are capsule cells, this preparation could provide a useful experimental model for studies on the interaction between neurones and their accompanying glial cell processes.

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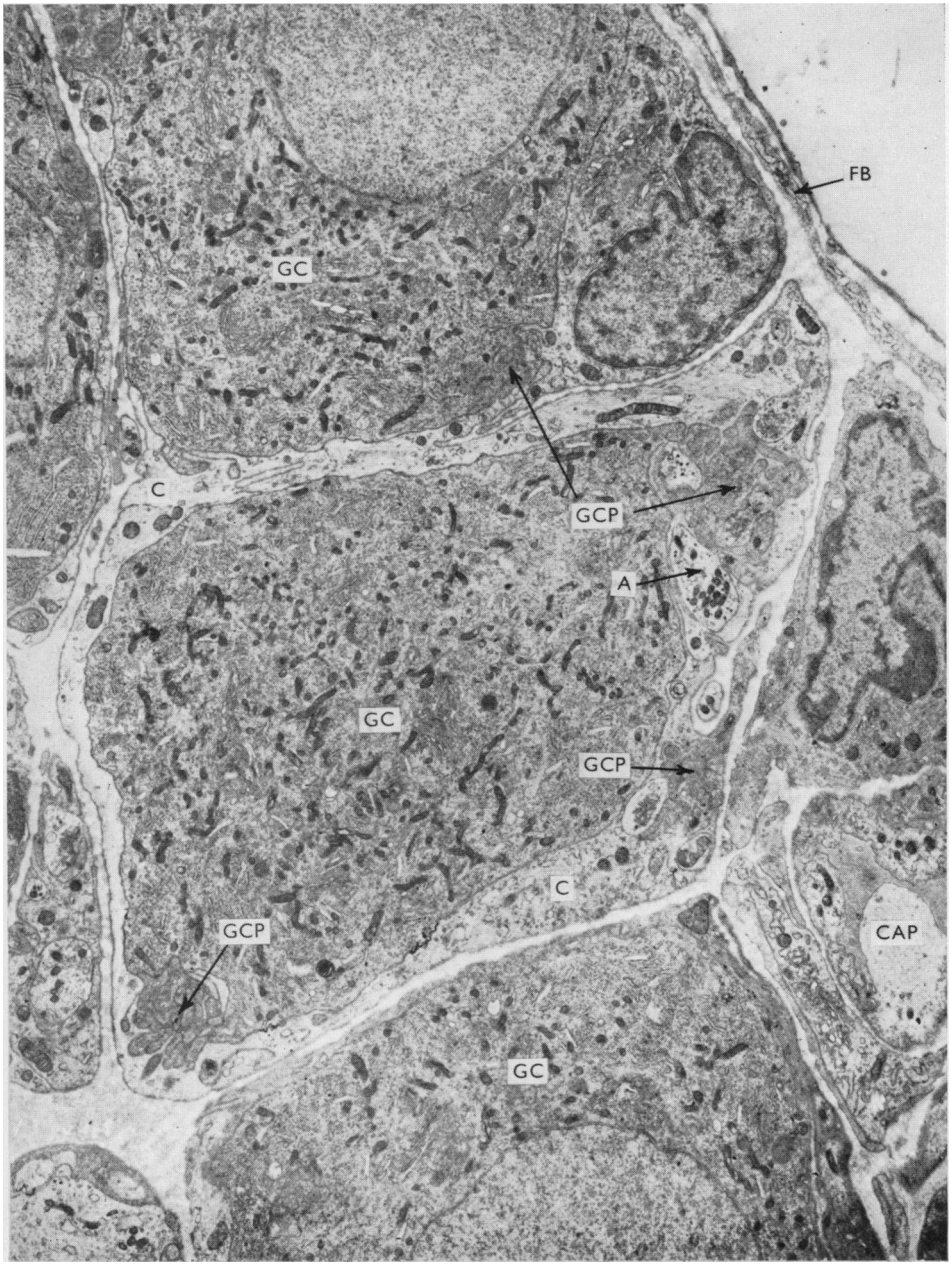
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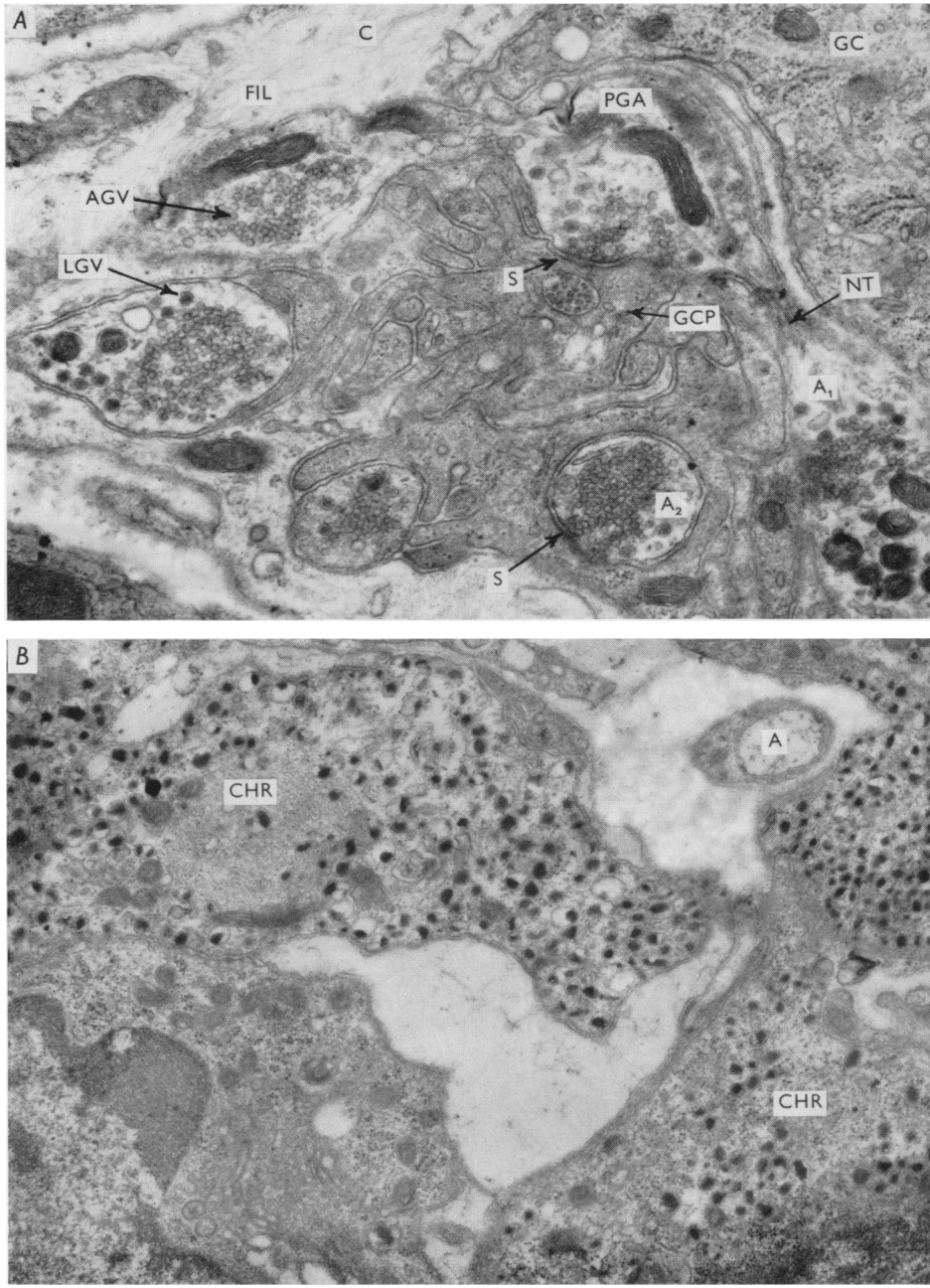
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EXPLANATION OF PLATES

PLATE 1

Low power electron micrograph of a portion of a pelvic ganglion. The ganglion cell bodies (GC), 20 μ in diameter, are surrounded by a thin capsule cell (C) and contain large numbers of mitochondria, ribosomes and endoplasmic reticulum. They appear to be more densely stained than axons and capsule cells. Between the ganglion cells, there are a few ganglion cell processes (GCP) and axons. Several preganglionic axons (A), surrounded by capsule cells, form a close relationship with the tuft-like ganglion cell processes. In the extracellular space, there is a capillary (CAP), and surrounding the ganglion there is a sheet of connective tissue and fibroblasts (FB). × 7000.

PLATE 2

A. A high power electron micrograph of a synapse of a preganglionic axon (PGA) with the tuft-like ganglion cell processes (GCP) from an adjacent section to that shown in Plate 1. The axons and ganglion cell processes are enveloped by the capsule cell (C) which contains fine filaments (FIL). The varicosities of the axons contain agranular vesicles (AGV), occasional large granular vesicles (LGV), and the narrow regions of the axon between the varicosities contain neurotubules (NT). The ganglion cell processes contain few cellular components and are more densely stained than the preganglionic axons. Serial sections (of which this figure and Plate 1 form a part) demonstrate continuity of the ganglion cell processes with the cell body and a continuity of the axons (A_1) with (A_2). At the synapse (S) of the preganglionic axon with the ganglion cell processes, the thickened pre- and post-synaptic membranes are 170 Å apart (at the outer surfaces of the two plasma membranes) and there is a higher concentration of agranular vesicles. × 26,000.

B. A portion of the processes of chromaffin cells (CHR) with vesicles (1000–1500 Å in diameter) containing a large densely staining granule (400–800 Å in diameter). Ribosomes, endoplasmic reticulum and mitochondria are also present. The chromaffin cells are usually found at regions where the large axon bundles enter or leave the ganglion. A small axon (A) is present. This ganglion was used for physiological studies and was fixed 5 hr after death of the animal. × 19,500.