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Effect of iontophoretic application of cholinergic agonists and their antagonists to guinea-pig pelvic ganglia

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Summary

1. The electrical activity of guinea-pig pelvic ganglion cells following iontophoretically applied cholinergic drugs, alone and during orthodromic nerve stimulation via the hypogastric nerve, has been recorded intracellularly.

2. Iontophoretic application of nicotine (Nic) and acetylcholine (ACh) reduced membrane resistance and caused a depolarization which in approximately 80% of cells led to the firing of action potentials. In the remainder, depolarization was unaccompanied by firing.

3. Iontophoretic application of Nic and ACh reduced or abolished the amplitude of successively evoked orthodromic responses.

4. ACh-induced depolarization, unlike that caused by tetanic stimulation, was not followed by a subsequent increase in the frequency of synaptic potentials.

5. Di-hydroβerythroidine (DHβE) and atropine (Atr) inhibited the response to both orthodromic stimulation and iontophoretic application of Nic and ACh.

6. There was no evidence for the existence of muscarinic receptors in guinea-pig pelvic ganglia. Iontophoretic application of muscarinic agonists alone and after tetanic stimulation of the hypogastric nerve produced no significant depolarization of the ganglion cell membrane.

Introduction

Present knowledge of the pharmacology of mammalian autonomic ganglia has been derived mainly from experiments using extracellular recording techniques in which the actions of drugs have been examined by adding them to the perfusing medium both *in vivo* and *in vitro* (see reviews by Volle, 1966, 1969 ; Tauc, 1967 ; Trendelenburg, 1967). Most mammalian ganglia consist of a mixed population of neurones (Hillarp, 1960) their associated glial or sheath cells and cells of a type intermediate between chromaffin and ganglion cells (Elfvin, 1963a, b ; Sjöstrand, 1965 ; Trendelenburg, 1967 ; Blackman, Crowcroft, Devine, Holman & Yonemura, 1969 ; Matthews & Raisman, 1969). Each of these types of cells is unlikely to

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respond to a particular drug in the same way (Shaw, Keogh & MacCallum, 1948 ; Shaw & MacCallum, 1949 ; Shaw, MacCallum, Dewhurst & Mainland, 1951) and measurements made with extracellular electrodes are indirect and unlocalized. This problem has been overcome by means of intracellular recording (Eccles, 1955, 1963 ; Ginsborg & Guerrero, 1964 ; Erulkar & Woodward, 1968 ; Blackman *et al.*, 1969 ; Libet & Tosaka, 1969). During such experiments the pharmacology of individual cells was studied by addition of drugs to the perfusing medium.

This paper deals with the response of single ganglion cells of the isolated pelvic plexus of the male guinea-pig to drugs placed in their immediate environment by iontophoresis from a five barrel micropipette under conditions similar to those used for pharmacological studies within the central nervous system (Phillis, 1965, and references cited).

A preliminary account of this work has been published (Muir & Yonemura, 1969).

Methods

The technique for dissection of the hypogastric nerve and the pelvic plexus from young male guinea-pigs (150–200 g) and for intracellular recording from individual ganglion cells was similar to that described by Blackman *et al.* (1969). The preparation was placed in 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. The solution was bubbled with 95% O_2 and 5% CO_2, warmed to 35–37° C and perfused through an isolated organ bath of less than 5 ml capacity at a rate of 2–3 ml/minute. The hypogastric nerve was stimulated by twin chlorided Ag electrodes. A Grass S4 or S8 stimulator was used throughout ; values of stimulus strengths in the text refer to the stimulator setting and not to the absolute value of the voltage between the stimulating electrodes. The pulse duration was 0·5 ms.

Five barrel micropipettes were made by fusing four Pyrex tubes (outer diameter 4 mm, wall thickness 1 mm) to an inner tube of the same diameter and were drawn in a vertical microelectrode puller taking care to ensure that the drawn out portion remained parallel to the axis. The tips were broken back under microscopical control to 6–8 μm diameter. The barrels were filled by boiling in distilled water which was then replaced by filtered aqueous solutions of drugs.

The following drugs were used (concentrations refer to the salts): acetylcholine chloride, ACh (1 M) ; acetyl-β-methylcholine, AβCh (0·5 M) ; atropine sulphate, Atr (0·8 M) ; dihydro-β-erythroidine, DHβE (0·5 M) ; nicotine hydrogen tartrate, Nic (0·1 M) ; and McNeil A-343 (0·1 M). The electrodes were stored for 24–36 h before use. The micropipette and the separate single recording electrode were held in ' parallel ' (Curtis, 1968) in a specially constructed holder. The single electrode (tip length 1·0–1·2 cm) was passed, under microscopical control, along the axis formed by two of the five barrels of the micropipette until the tip of the single electrode projected 60–100 μm. This distance and the transverse distances between the electrodes (less than 10 μm) were thus maintained unchanged during penetration of the tissue, a pre-requisite to ensure that extracellularly administered drugs diffused to the impaled cell.

Drugs were applied iontophoretically from the five barrel micropipettes using a D.C. polarizer and retaining voltage (0·5 V) as described by Curtis (1964). The amount of drug administered is expressed in nanoamps (nA, 10^{-9}A). In most cases, the amount of current used to eject a compound ranged from 16 to 70 nA, but in a few instances, currents of 100–200 nA were used. A potential in the depolarizing direction, due to resistive coupling between the single electrode and the micropipette, always accompanied the passage of cationic, ejecting current. For currents less than 70 nA it was less than 10 mV and for currents 100–200 nA it ranged from 15 to 45 mV. These coupling potentials were measured by ejecting the various compounds after the single recording electrode was withdrawn from the cell.

To determine whether the iontophoretic application of a drug changed the input resistance of the membrane, the steady state change in membrane potential for a constant current pulse was determined before, during and after application of the drug. The current was passed by the recording electrode either using a Wheatstone bridge circuit as described previously (Martin & Pilar, 1963 ; Hashimoto, Holman & Tille, 1966) or a solid state electrometer developed by Fein (1966).

Results

The results described here were obtained from 186 ganglion cells which responded to direct intracellular stimulation with one or more action potentials. Other cells were also impaled during these experiments which failed to respond to direct stimulation. These may have been Schwann cells or the glial cells surrounding ganglion cell soma (Blackman & Purves, 1969).

Responses to acetylcholine and nicotine

Iontophoretic application of ACh and Nic caused depolarization. In approximately 80% of cells, this led to the firing of action potentials of the spike form. Figure 1 shows examples of the action of ACh and Nic on six different cells and

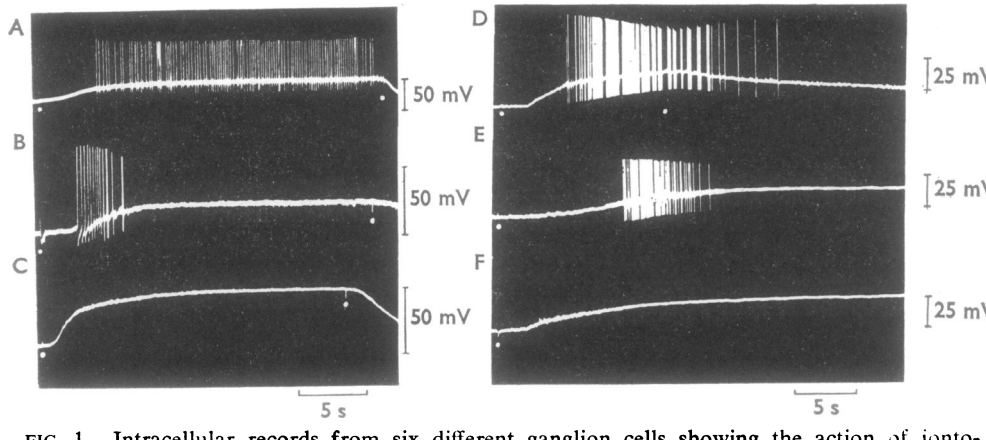

FIG. 1. Intracellular records from six different ganglion cells showing the action of iontophoretically applied ACh and Nic: A, ACh 100 nA ; B, ACh 32 nA ; C, ACh 68 nA ; D. Nic 56 nA ; E, Nic 52 nA ; F, Nic 28 nA. In E and F Nic was applied for 45 seconds. In this and other figures, the duration of iontophoretic application of the drug is indicated by the time interval between the white dots.

illustrates the three types of response observed throughout these experiments: (i) continuous firing of spikes throughout the period of application of ACh (Fig. 1A) and Nic (Fig. 1D); (ii) a burst of spikes of progressively decreasing amplitude followed by maintained depolarization (Fig. 1B and E); (iii) depolarization alone (Fig. 1C and F).

In some cells, 10 nA of either ACh or Nic were sufficient to evoke spike discharge throughout the period of application. Depolarization and spike discharge, if present, subsided after the drug had been turned off.

The pattern of spike discharge was related to the degree of depolarization produced. A period of spike discharge could be maintained continuously throughout the period of application of the drug (Fig. 1A and D). With larger amounts of depolarization the discharge was transient (Fig. 1B and D) or even absent (Fig. 1C and F) but the depolarization was maintained. The critical amount of depolarization beyond which spike discharge was not continuous was, after correcting for the coupling potential, approximately 25 mV. With Nic, firing was usually abolished within 1–2 minutes. This may have been due to the difficulty in maintaining the current used to pass Nic for long periods at levels which did not cause a large depolarization.

The level of depolarization associated with the onset of the spike discharge induced by ACh or Nic was similar to that required to initiate an action potential by direct stimulation (10–20 mV in different cells).

In a typical experiment demonstrating the relationship between drug concentration and membrane depolarization in which a series of responses to ACh was recorded from one cell, large doses of ACh (greater than 140 nA) depolarized the membrane beyond the firing zone. The change in membrane potential, measured 1–1·5 min after the onset of depolarization, increased with increasing doses of ACh. The peak depolarization induced by ACh reached approximately 30 mV. This cell had a resting membrane potential of −40 mV; thus the resting membrane potential at the peak of the depolarization induced by ACh was −10 mV. This value agrees with that found by Nishi & Koketsu (1960) and Blackman, Ginsborg & Ray (1963) for the membrane potential of frog sympathetic ganglion cells exposed to supramaximal doses of ACh and also agrees with estimates of the reversal potential for the excitatory synaptic potentials observed in pelvic ganglion cells of the guinea-pig (Crowcroft, Muir & Szurszewski, 1970).

The latency of onset of the depolarization to both ACh and Nic varied from several hundred ms to 10 seconds. It is difficult to explain some of the long latencies observed in response to relatively larger doses of either agonist in terms of simple diffusion (e.g. Fig. 1D). Since the distance between the tip of the recording electrode and the source of drug was about 60 μm the concentration of ACh or Nic should have begun to build up in the region of the cell membrane in less than 0·5 s (del Castillo & Katz, 1955). It is possible that the glial cells surrounding the ganglion cell soma may represent a diffusion barrier of variable thickness.

ACh and Nic were also applied for successive brief (10–30 s) periods. The result for Nic is shown in Fig. 2. A slight reduction in the latency of successive responses was observed but the frequency and amplitude of the spikes were maintained. When repetitive firing throughout the first period of application of either drug

occurred, spike discharge was also sustained during repeated successive similar doses of Nic (seven applications, 20 nA) and ACh (eight applications, 16 nA) given at 20 and 10 s intervals.

In several experiments ACh and Nic were applied for much longer periods (up to 4 min). There was no decrease in the level of depolarization during the steady application of either agonist. In these experiments, repetitive spike discharge in response to iontophoretic application of ACh was observed in certain cells for up to 3 minutes. In one experiment Nic was applied continuously for 7 minutes. Although on this occasion there was a small apparent decrease in depolarization, it was not possible to distinguish it from spontaneous fluctuations in membrane potential.

As pointed out above, a discharge of spikes in response to both ACh or Nic was observed in 80% of the cells which responded to direct stimulation with one or more spikes. In the remaining 20% of cells, although both ACh and Nic caused depolarization, no firing of spikes occurred (e.g. Fig. 1C and F). Increasing doses of either drug increased the amount of depolarization but failed to produce a spike

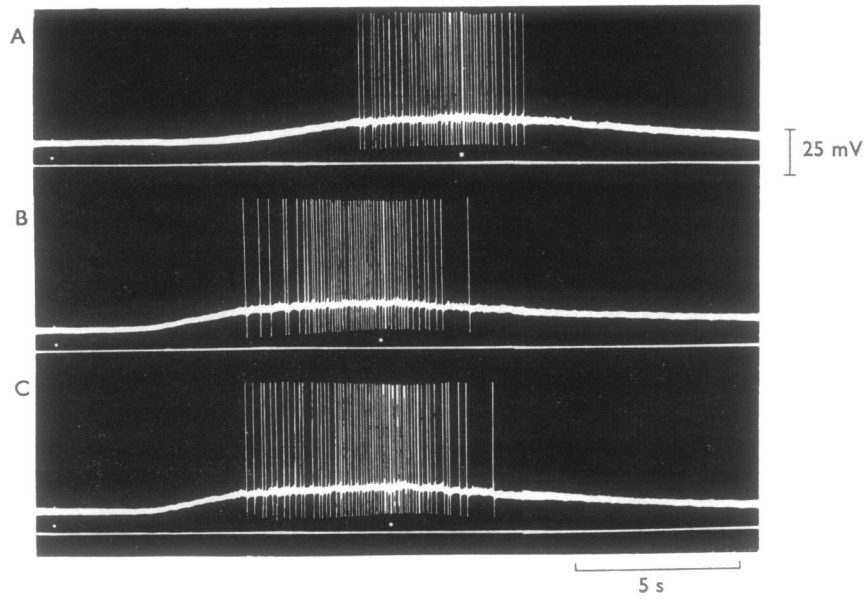

FIG. 2. Intracellular response of a ganglion cell to three successive applications of Nic (20 nA) at 20 s intervals.

TABLE 1. *Comparison of membrane characteristics of firing and non-firing cells following iontophoretic application of ACh and Nic*

Membrane characteristic	Cell firing to ACh and Nic	Cell not firing to ACh or Nic	95% Confidence limits for true difference
Resting membrane potential (mV)	58·3 (30)	52·7 (26)	6·25 to −4·05
Amplitude of action potential (mV)	84·0 (16)	67·9 (13)	80·68 to −48·48
Input resistance, R input ($\times 10^6\ \Omega$)	39·0 (15)	40·1 (14)	19·10 to −21·90
Time constant (ms)	5·3 (12)	5·4 (14)	1·03 to −0·51
Threshold current ($\times 10^{-10}$A)	2·9 (13)	2·7 (10)	1·62 to −1·45

Number of observations given in brackets.

discharge. To see whether there was any difference in the characteristics of firing and non-firing cells their properties were compared. As shown in Table I there was no significant difference between the two groups of cells with respect to their resting membrane potentials, input resistance and time constant, the amplitude of their action potentials or the threshold current needed to evoke a spike by direct stimulation.

When brief depolarizing current pulses of suprathreshold intensity were passed across the cell membrane during depolarization of a non-firing cell by ACh and Nic, spikes could be elicited throughout the onset of depolarization and during the period of maintained depolarization, provided this was not greater than about 25 mV. This result suggests that the absence of spikes in response to the application of Nic and ACh in some cells was probably due to the ability of these cells to accommodate to relatively slow rates of depolarization. There was no obvious correlation between the rate of depolarization induced by ACh and Nic in firing and non-firing cells and it may be that the tendency to accommodate varied from one cell to another. Following prior treatment with eserine, spikes could be elicited by ACh in otherwise non-firing cells (Szurzewski, personal communication).

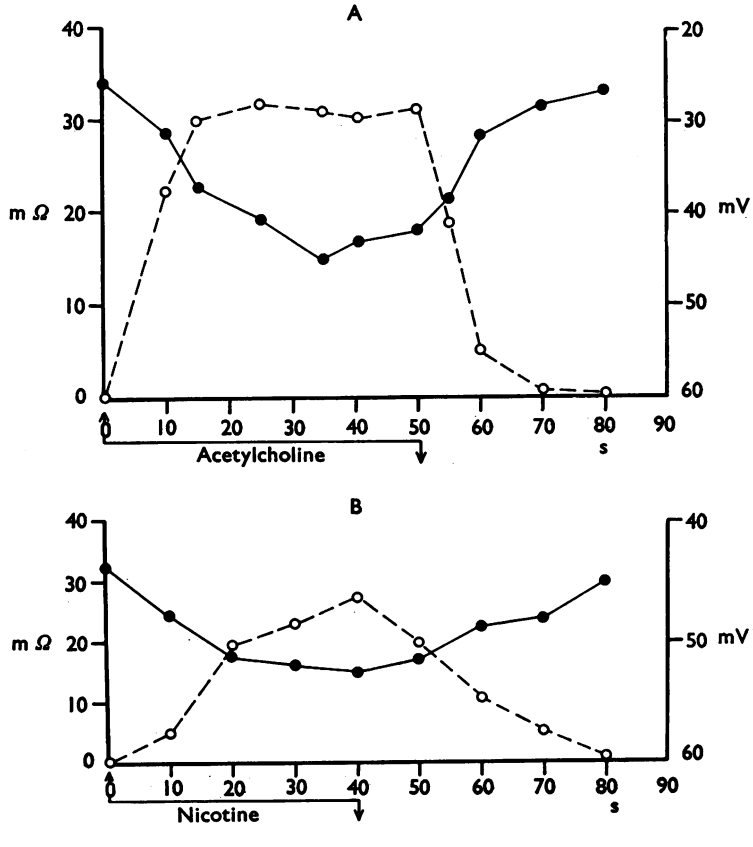

FIG. 3. Changes in membrane resistance (●) and potential (○) during and after the iontophoretic application of ACh (130 nA) in A and Nic (180 nA) in B. No correction was made for the coupling potential in this experiment.

Effect of ACh and Nic on membrane properties

In both firing and non-firing cells, ACh and Nic rapidly and reversibly reduced membrane resistance (Fig. 3). Changes in membrane resistance reached their maximal values at the same time as the maximum level of depolarization.

Depolarizing currents of intensity sufficient to evoke a spike during the control period were passed across the cell membrane during the action of ACh. As already pointed out, such pulses continued to evoke spikes during the onset of depolarization in non-firing cells. But when the membrane potential had decreased to a level at which spikes no longer occurred in a firing cell such pulses were unable to evoke spikes. During repolarization after the drug had been turned off, brief depolarizing current pulses were found to be capable of initiating spikes once again, as soon as the membrane potential repolarized beyond the critical level at which firing was blocked during depolarization.

Responses to orthodromic stimulation

In general, cells responded to orthodromic stimulation of the hypogastric nerve with excitatory synaptic potentials which were of sufficient amplitude to trigger one or more spikes. Iontophoretic application of either ACh or Nic altered the response to orthodromic nerve stimulation. In some cells such as that providing the result in Fig. 4, the spikes in response to orthodromic stimulation were observed throughout the period of application of Nic (52 nA) although their amplitude was reduced to about 20 mV. In other cells, the amplitude of the action potential evoked by orthodromic nerve stimulation was first reduced and then abolished leaving only the synaptic potential. In Fig. 5, the spike evoked by orthodromic stimulation failed about 60 s after the onset of the application of Nic, leaving a small synaptic potential. After turning off Nic, the amplitude of the synaptic potential increased as the level of membrane potential increased and after 30 s evoked a spike once again. (Note that no correction has been made for the coupling potential which was less than 10 mV in the records of Figs. 4 and 5.)

There was no evidence for a decrease in transmitter action in either of these cells. In Fig. 4B and C, the level of depolarization following the spike was unaltered. Even if allowance is made for the coupling potential, the fact that the orthodromic response is elicited at a lower resting membrane potential than that of the control response could easily explain the change in its configuration.

Locus of drug-induced depolarization

Controversy persists following the suggestion (Koelle 1961 ; Volle & Koelle, 1961) that in the cat sympathetic ganglion certain drugs release ACh from preganglionic nerves, which in turn causes the depolarization of the postganglionic cells. If this occurred in the guinea-pig, and the quantal release of ACh was small, then drug-induced depolarization might take the form of a series of synaptic potentials as suggested by Ginsborg & Guerrero (1964). There was no evidence that the depolarization induced by ACh or Nic was discontinuous. On the contrary, when the time course of the response to these drugs was examined at high gain it was clear that the depolarization was completely smooth in onset. This result confirms that of Ginsborg & Guerrero (1964).

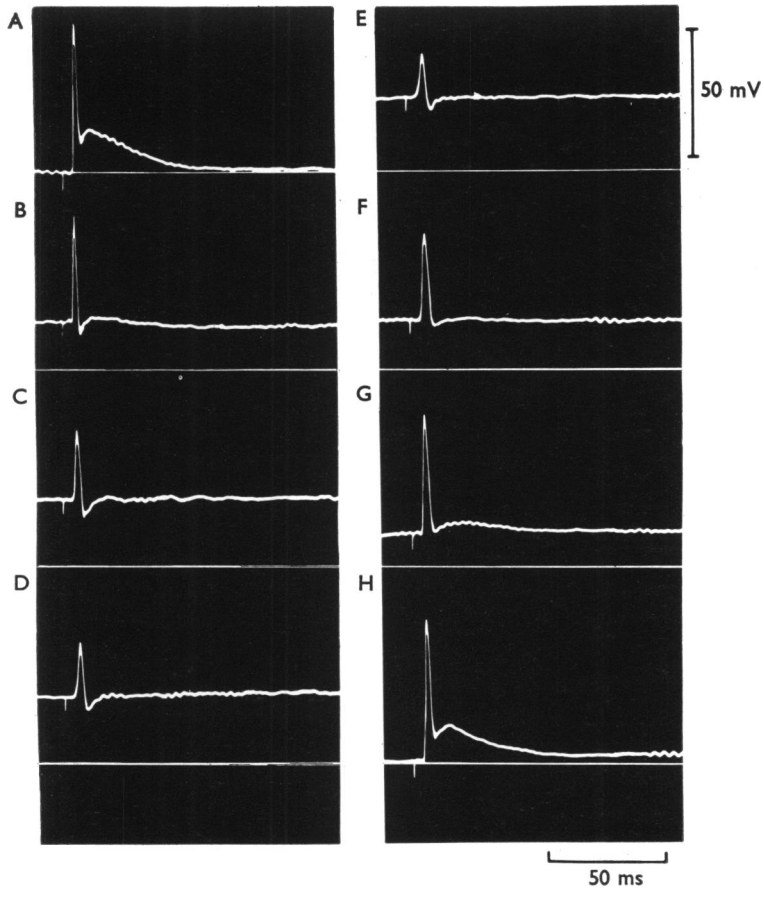

FIG. 4. Intracellular records showing responses of a ganglion cell to stimulation of the hypogastric nerve (7 V, 0·5 ms, 0·7 Hz) before (A) and 10 s (B), 40 s (C), 50 s (D), and 60 s (E) after the onset of Nic (52 nA). Records F–H were taken 10 s, 40 s, and 2 min, respectively, after the Nic current was turned off. The thin white lines indicate the original level of the resting membrane potential. No correction was made for the coupling potential in records B–E.

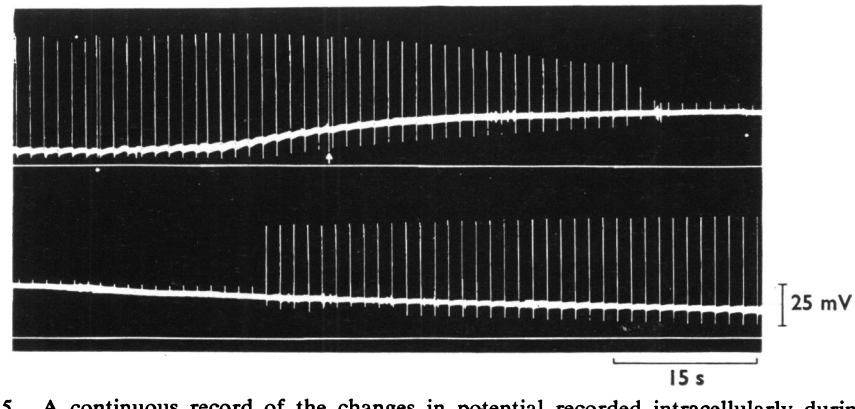

FIG. 5. A continuous record of the changes in potential recorded intracellularly during and after the application of 40 nA of Nic for 30 s followed by 200 nA of Nic (onset of larger current indicated by arrow) to a ganglion cell. Stimulation of the hypogastric nerve (7 V, 0·5 ms) was at a frequency of 0·7 Hz throughout.

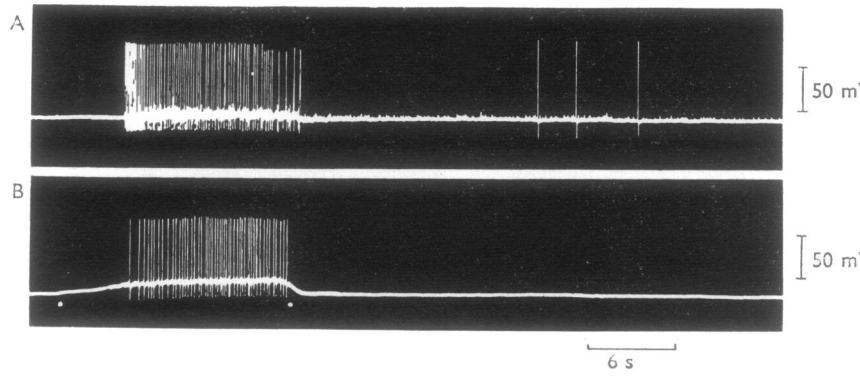

FIG. 6. A, Intracellular response of a ganglion cell to repetitive stimulation of the hypogastric nerve (15 V, 0·5 ms) for 12 s at 10 Hz. Note the discharge of small synaptic potentials following the cessation of stimulation. B, Comparable discharge of spikes in response to ACh (95 nA) for 12 seconds.

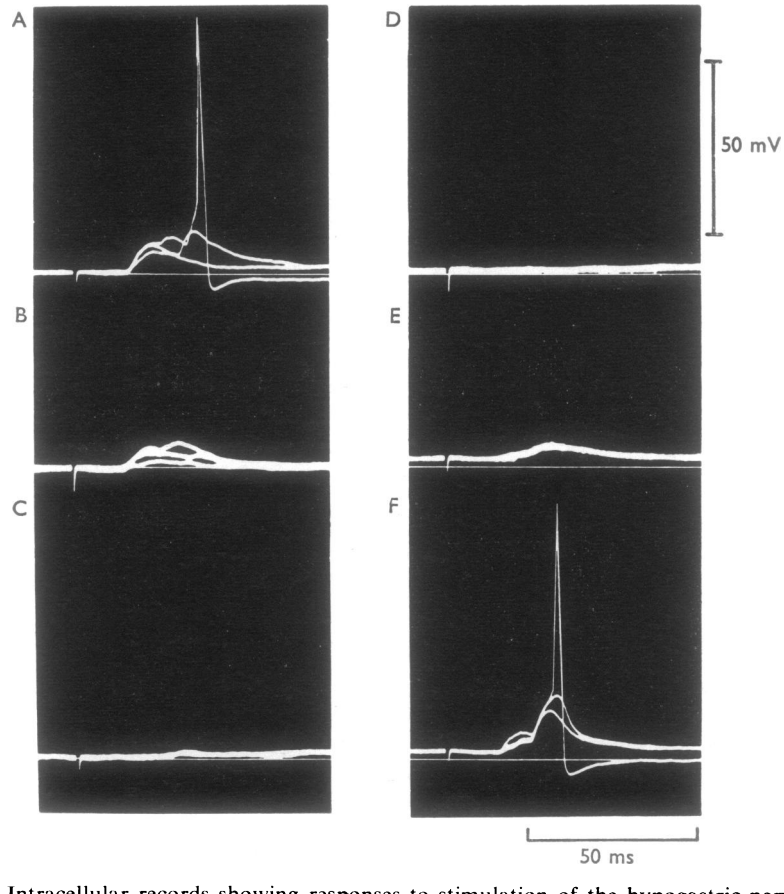

FIG. 7. Intracellular records showing responses to stimulation of the hypogastric nerve (60 V, 0·5 ms, 0·7 Hz) before (A) and 10 s (B), 20 s (C) and 30 s (D) after the onset of iontophoretic application of DHβE (50 nA). Each record is three superimposed responses to single stimuli. Records E and H were taken 4 min and 15 min, respectively, after the drug was turned off. The thin white lines indicate the original level of the resting membrane potential.

Spontaneous synaptic potentials were rarely seen in the absence of nerve stimulation in our preparation. However, as described previously (Blackman *et al.*, 1969), their frequency was markedly increased following tetanic stimulation of the hypogastric nerve (Fig. 6A). Following a similar train of action potentials induced by ACh there were no synaptic potentials (Fig. 6B).

Effect of DHβE

DHβE completely inhibited responses to orthodromic stimulation and to iontophoretically applied ACh and Nic. In the experiment shown in Fig. 7, DHβE (52 nA, 30 s) abolished the spike evoked by single maximal orthodromic stimuli after 10 s, leaving an excitatory post-synaptic potential (Fig. 7B) which disappeared approximately 20 s later (Fig. 7D). Following withdrawal of DHβE, the sequence was reversed, the form of the original response being restored in approximately 15 min (Fig. 7F). Figure 8 shows the action of DHβE (80 nA, 5 s) on Nic-induced firing. Approximately 6 min after withdrawal of DHβE the firing pattern of Nic (20 nA, 20 s) had recovered (Fig. 8D). Qualitatively similar results were obtained with ACh.

No change in the membrane resistance occurred during the passage of DHβE.

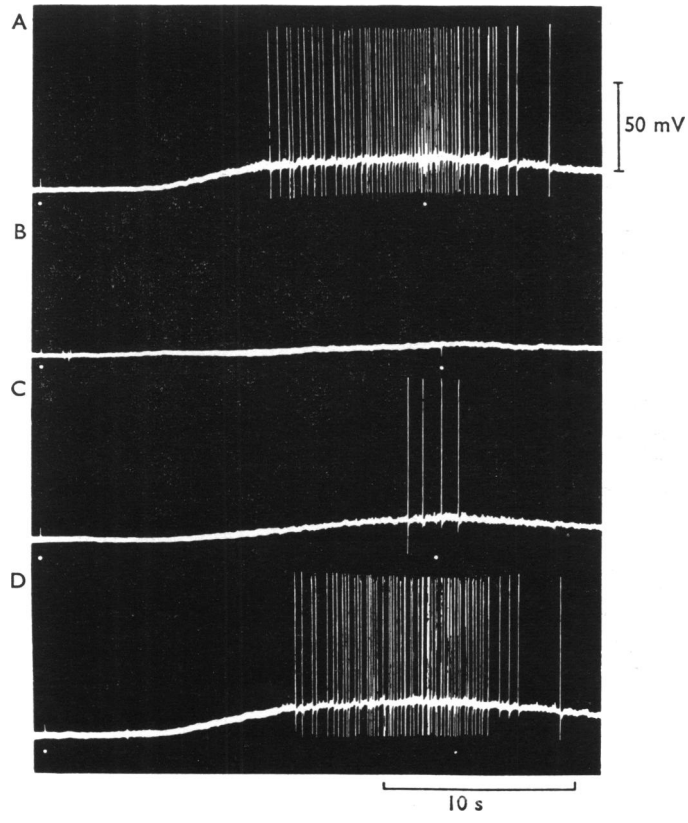

FIG. 8. Intracellular response of a ganglion cell to Nic (20 nA) before (A) and 30 s (B) after the onset of DHβE (52 nA) applied iontophoretically. Records C and D were taken 40 s and 2 min, respectively, after DHβE was turned off.

Effect of Atr

Atr, like DHβE, reversibly abolished firing in response to orthodromic stimulation and iontophoretically applied ACh and Nic. Compared with DHβE, however, the intensity and duration of application of Atr necessary to abolish these responses and the time taken for their recovery (10–15 min) were greater. Figure 9 illustrates the effect of Atr (180 nA, 10 min) on responses to single maximal orthodromic stimuli. The depolarization following the spike (Fig. 9A) which was probably due to the continued action of the transmitter, was abolished first (30 s, Fig. 9B). The membrane was depolarized (Fig. 9C) and changes in the shape and amplitude of the action potential became evident. Approximately 3 min later, the spike was abolished leaving only a synaptic potential (Fig. 9E) which itself disappeared approximately 2 min later. Membrane potential and spike recovered to within a few millivolts of their original values 30 min after the Atr was turned off.

Since relatively large currents were used in these experiments, the true value of the depolarization could not be ascertained. It is difficult to delineate precisely

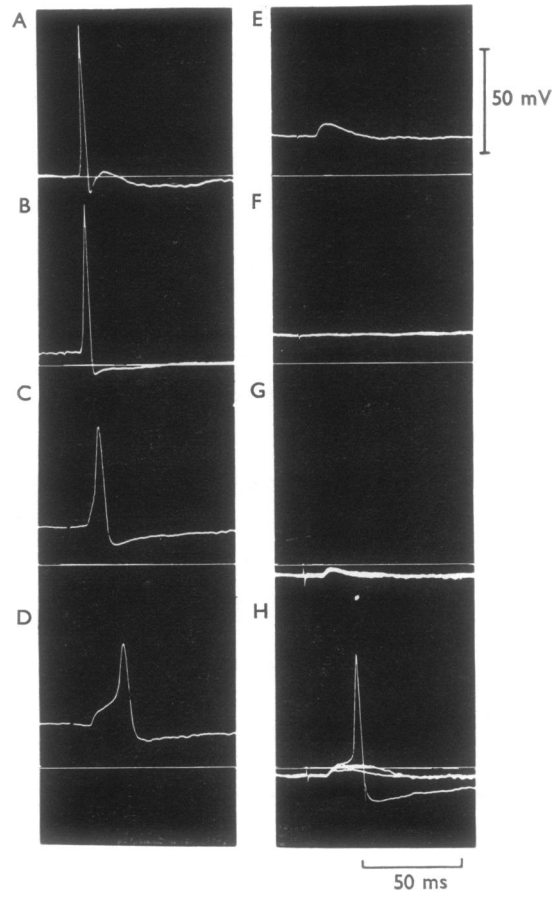

FIG. 9. Intracellular records showing response of a ganglion cell to stimulation of the hypogastric nerve (10 V, 0·5 ms, 1 Hz) before (A) and 30 s (B), 130 s (C), 170 s (D) and 190 s (E) after the onset of Atr (180 nA). Records F, G and H were taken 10, 15 and 30 min, respectively, after Atr was turned off. The thin white lines indicate the original level of resting membrane potential. Records G and H were of three successive superimposed responses.

the role of Atr in these observations. Two aspects of its action may be involved. Atr may have raised the threshold for spike initiation by the synaptic potential before the latter was itself abolished or it could have progressively reduced the size of the synaptic potential without change in the spike threshold. The progressive reduction in spike amplitude shown in Fig 9B, C and D could have been due, in part, to inactivation of inward current as a result of depolarization, but is more likely to be due to a direct stabilizing action of Atr on the ganglion cell membrane (Curtis & Phillis, 1960). Atr also blocked firing induced by ACh and Nic (Fig. 10) thus confirming the drug's ability to interact with nicotinic receptors located post-synaptically.

Muscarinic receptors

Iontophoretic application of two muscarinic agonists, AβCh and McNeil compound A-343, failed to evoke spikes in cells which responded in this way to ACh and Nic. A small apparent depolarization was observed in response to very large currents (greater than 200 nA) but this could not be distinguished from the coupling potential.

In several mammalian ganglia, including the superior cervical ganglion of the cat, responses to muscarinic agonists were potentiated following repetitive preganglionic nerve stimulation (Trendelenburg, 1967). We therefore tested the action of AβCh before, during and after repetitive stimulation of the hypogastric nerve at frequencies ranging from 10 to 40 Hz. A series of typical responses to repetitive stimulation is shown in Fig. 11A, B and C. The train of spikes recorded during stimulation was followed by a period of hyperpolarization, the duration of which

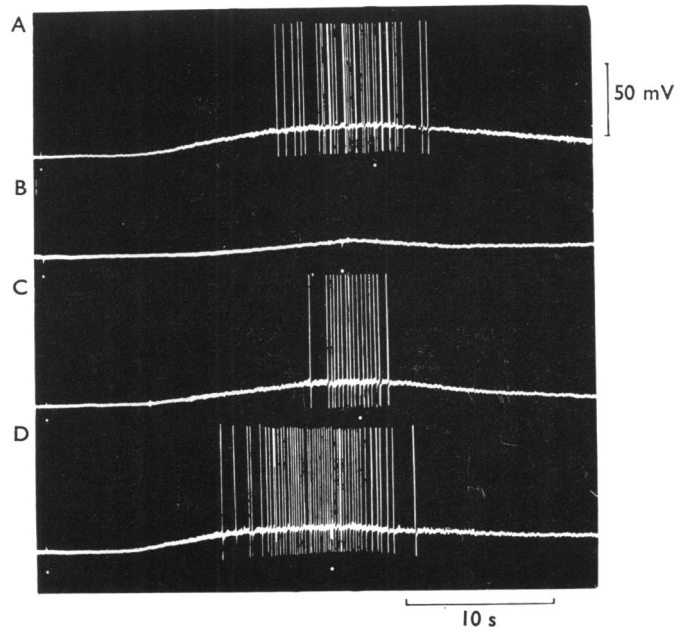

FIG. 10. Intracellular response of a ganglion cell to Nic (20 nA) before (A) and 2 min (B) after the onset of Atr (48 nA). Records C and D were taken 35 s and 6 min, respectively, after Atr was turned off. Both drugs were applied iontophoretically.

increased with increasing frequencies of stimulation. As described previously, a discharge of small spontaneous synaptic potentials was also observed which occasionally gave rise to a spike. AβCh (92 nA) had no effect on the nature of this response, nor did it cause depolarization or the firing of spikes following the tetanus.

Figure 11D, E and F, shows responses to repetitive orthodromic stimulation in the presence of DHβE (24 nA) recorded from the same cell as that of Fig. 11A, B and C. Although excitatory synaptic potentials were recorded in the presence of the drug, no hyperpolarization occurred after cessation of stimulation. The hyperpolarization following stimulation in Fig. 11A, B and C would appear to be a consequence of the initiation of spikes.

In a further series of experiments in which the effects of repetitive stimulation of the hypogastric nerve were compared with the effects of repetitive direct stimulation, in the great majority of cells both methods of stimulation gave rise to the same form of after potentials (Crowcroft & Szurszewski, personal communication). Slow synaptic potentials of the type described by Eccles & Libet (1961) which are blocked by Atr do not appear to be an obvious feature of the response of pelvic ganglion cells to stimulation of the hypogastric nerve.

Discussion

The observed actions of ACh, Nic and DHβE are in keeping with the classic hypothesis that ganglionic transmission is mediated by nicotinic receptors. Only the depolarizing agonist actions of ACh and Nic were seen in this study. Depression of both spikes and synaptic potentials evoked by orthodromic stimulation occurred during depolarization by both drugs but this effect could be explained by the depolarization itself. Failure of the spike evoked by orthodromic stimulation occurred at a level of membrane potential similar to that which blocked the spike in response to direct stimulation.

We may have failed to observe the antagonist actions of ACh and Nic (Paton & Perry, 1953 ; Ginsborg & Guerrero, 1964) as a result of the relatively short periods

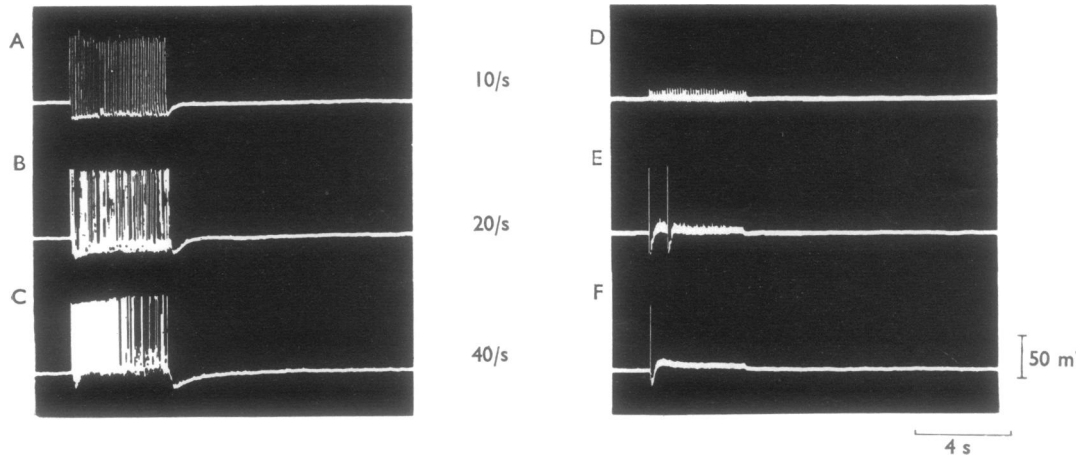

FIG. 11. A, B and C, successive intracellular responses from a ganglion cell to repetitive stimulation (14 V, 0·5 ms) for 4 s at 10 Hz, 20 Hz and 40 Hz, respectively. D, E and F recorded 5 min after the application of DHβE, 24 nA (parameters of stimulation as in A, B and C).

of exposure to these drugs. However, it is interesting that the maximum level of depolarization in response to ACh and Nic was well maintained in all our experiments for periods of up to 3 and 7 min, respectively, whereas Ginsborg & Guerrero found that the membrane potential started to return to normal within 30 s in some cells (Ginsborg & Guerrero, 1964, Fig. 5B). It is possible that the time course of onset of the late, non-depolarizing, blocking action of ACh and Nic may be considerably slower in guinea-pig pelvic ganglia than in frog sympathetic chain. Alternatively, this difference may be due to the different ways in which the drugs were applied. In Ginsborg & Guerrero's experiments, drugs were added to the solution in the bath whereas in our experiments they were applied locally.

The observed changes in membrane resistance are in agreement with the findings of Ginsborg & Guerrero (1964). Our estimate for the reversal potential for ACh also agrees with their result for the frog sympathetic chain ganglia and with those of Crowcroft *et al.* (1970). No changes in membrane potential or resistance were observed during the application of DHβE, confirming its purely antagonist action against ACh applied iontophoretically or against ACh released from presynaptic nerve terminals by orthodromic nerve stimulation.

We were surprised to discover that Atr blocked both the action of Nic and responses to preganglionic nerve stimulation. Admittedly, large currents were used to eject atropine in some of these experiments but currents of similar magnitude have been used in studies on the central nervous system (see Curtis & Phillis, 1960). Our results show that high, local concentrations of Atr have complex effects on ganglion cells. Nicotinic receptors may be blocked and the threshold for initiation of an action potential is increased. These actions of Atr appear to be identical with its unspecific depressant effect on cortical neurones (Krnjević & Phillis, 1963).

One of the aims of this study was to search for evidence of muscarinic receptors in these ganglia. This we failed to find. The absence of muscarinic receptors confirms previous findings (Bentley, 1968) and would support the hypothesis that the pelvic ganglia function mainly as relay and distributing centres and not as an integrating centre (Blackman *et al.*, 1969).

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