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THE EFFECTS OF PHYSOSTIGMINE ON SYNAPTIC TRANSMISSION IN THE INFERIOR MESENTERIC GANGLION OF GUINEA-PIGS

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SUMMARY

1. Synaptic potentials were recorded with intracellular electrodes from cells in the inferior mesenteric ganglion of the guinea-pig.

2. Half-widths of the synaptic potentials recorded fell into two groups: type L cells had long synaptic potentials (11·6–15·2 msec) and low thresholds (14·6 mV mean), type S cells had short synaptic potentials (6·1–9·3 msec) and high thresholds (29·9 mV mean).

3. Physostigmine ($1{\cdot}2 \times 10^{-6}$ M) caused a significant increase in the half-width of both types of synaptic potential.

4. Physostigmine caused a significant increase in the half-width of spontaneous synaptic potentials and an increase in their amplitude.

5. Repetitive preganglionic stimulation, in the presence of physostigmine, led to a marked and prolonged depolarization in all cells. In most cells repetitive spontaneous firing of action potentials was then observed. This effect was blocked by atropine ($1{\cdot}4 \times 10^{-7}$ M).

6. The effect of atropine on the half-width in a physostigmine-treated cell was inconsistent: although synaptic potentials in some cells were slightly shortened their half-widths were always greater than the control.

7. It is concluded that cholinesterase plays a role in limiting the time course of the synaptic potential, by limiting the duration of action of acetylcholine.

INTRODUCTION

The presence of acetylcholinesterase (AChE) has been demonstrated in mammalian sympathetic ganglia by many authors (e.g. Feldberg & Gaddum, 1934; Glick, 1937; Rosenblueth & Simeone, 1938; McLennan, 1954; Koelle & Koelle, 1959). Feldberg and his colleagues (Feldberg & Gaddum, 1934; Feldberg & Vartiainen, 1934*a*, *b*) found that in the presence of physostigmine, acetylcholine (ACh) was released by preganglionic stimulation, and that physostigmine potentiated the effects of

both submaximal stimulation and applied ACh in the superior cervical ganglion of the cat. Eccles (1934, 1935, 1937) reported to the contrary, that physostigmine had little or no effect on the properties of transmission in the same preparation. Recent studies using intracellular recording techniques in frog ganglia have yielded inconclusive or contradictory results (Blackman, Ginsborg & Ray, 1963*a*; Dennis, Harris & Kuffler, 1971). The results of Bennett & McLachlan (1972 *a*, *b*) suggest that the role of AChE in mammalian ganglia is to ensure a supply of choline, whose uptake into preganglionic terminals is essential for the synthesis of ACh. The experiments described below are an attempt to re-examine the role of AChE in determining the duration of the synaptic potential due to a single stimulus. Some observations on the effects of repetitive stimulation were also made.

Crowcroft & Szurszewski (1971) showed that each ganglion cell in the inferior mesenteric ganglion of guinea-pigs has many presynaptic fibres terminating on it and that the synaptic potentials produced by these fibres are nearly all subthreshold. This preparation was used to study the effects of physostigmine on the time course of minimal synaptic potentials evoked by the stimulation of a single preganglionic fibre.

A preliminary report of these experiments was communicated to the Australian Physiological and Pharmacological Society (Bornstein, 1973).

METHODS

Intracellular records were made from the inferior mesenteric ganglion (inf. mes. gang.) of guinea-pigs, using techniques similar to those described by Crowcroft & Szurszewski (1971). Preganglionic fibres in the ascending mesenteric nerve were stimulated with twin point Pt electrodes; a stimulus duration of 0·1 msec was used in all experiments and the stimulus strength was varied.

When the micro-electrode entered a cell there was a sharp negative deflexion of the recorded potential. Penetration of a cell was often accompanied by a burst of action potentials and this 'injury discharge' lasted for up to 15 sec before the membrane potential settled to a stable resting potential.

The results described below have been limited to penetrations which conformed with the following criteria.

1. The resting potential was stable.
2. Passage of a depolarizing current pulse through the micro-electrode caused an action potential of at least 65 mV.
3. Stimulation of the ascending mesenteric nerve gave at least one synaptic potential per stimulus (when an adequate stimulus strength was used).
4. The threshold of the cell was greater than 10 mV (this criterion could not be used in the presence of physostigmine – see below).

Some cells were held for up to 3 hr but recording periods of about 25 min were more common. The quality of the penetration, according to the above criteria, often improved markedly during the first 10 min of recording. It was found that penetrations could be held with electrodes varying widely in resistance (25–150 MΩ) and thus presumably varying in tip diameter; a more critical factor appeared to be how securely the preparation was pinned out.

Drugs were added to the inflow system so that a constant concentration in the organ bath would be achieved after 5–10 min. Drugs used were physostigmine sulphate and atropine sulphate. Concentrations were $1{\cdot}2 \times 10^{-6}$ M and $1{\cdot}4 \times 10^{-7}$ M respectively in all experiments. Recordings were made at least 15 min after the addition of physostigmine and 25 min after the addition of atropine.

RESULTS

Synaptic potentials

Ganglion cells responded to stimulation of the ascending mesenteric nerve in a manner which varied with stimulus strength in a discontinuous way. As stimulus strength was increased first one then two and then further synaptic potentials were observed. Each synaptic potential was elicited when the strength of the stimulus reached a distinct threshold below which the synaptic potentials never occurred and above which they were nearly always observed (sometimes, with very small synaptic potentials intermittent 'failures' were observed). The latency of each new synaptic potential was constant, and was different for each synaptic potential. The lowest threshold or minimal synaptic potential usually (but not always) had the shortest latency, and the latency of subsequent synaptic potentials usually increased as the threshold of the synaptic potential increased. This was interpreted to mean that additional fibres were being recruited as the stimulus strength was increased (Crowcroft & Szurszewski, 1971). In the experiments described here, unless otherwise stated, measurements were made on synaptic potentials of minimal threshold.

A single 'minimal' synaptic potential did not normally lead to an action potential and summation of several synaptic potentials was required to excite most ganglion cells. In six cells (out of seventy-two) stimulation of a single presynaptic fibre led to 'strong' synaptic action similar to that described by Blackman, Crowcroft, Devine, Holman & Yonemura (1969) and Crowcroft & Szurszewski (1971). Stimulation of a 'strong' presynaptic fibre always produced an action potential which was followed by depolarization probably due to continuing action of the ACh. This depolarization masked the normal hyperpolarization following the action potential. Thus a strong synaptic potential is considered to be the response of the ganglion cell to the release of a large amount of ACh, i.e. the cell is depolarized well beyond threshold (Blackman, Ginsborg & Ray, 1963*a*, *b*). Cells with 'strong' synaptic potentials have not been included in this analysis.

The amplitude of minimal synaptic potentials varied from 1·0 to 25·0 mV (mean 7·6 mV). In individual cells, successive stimuli at a constant strength gave rise to synaptic potentials whose amplitude sometimes fluctuated by

up to 5 mV. Fig. 1 shows synaptic potentials from twelve successive stimuli of constant strength at a frequency of 1 Hz; the fluctuation in amplitude is obvious and is similar to the fluctuations in the amplitude of

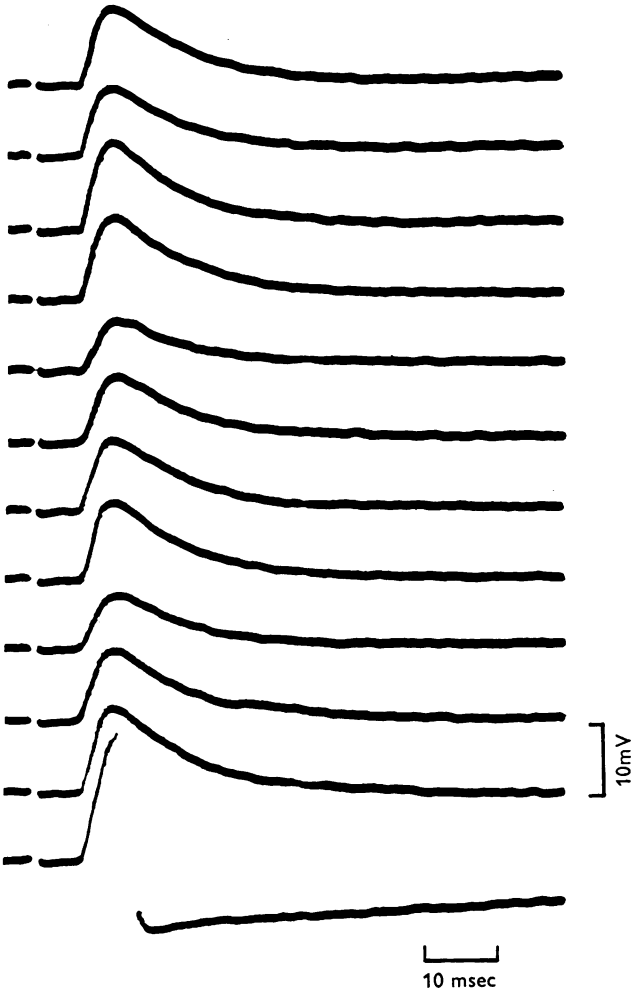

Fig. 1. Successive responses (minimal synaptic potentials, see text) to repetitive stimulation at 1 Hz (the last response exceeded threshold for an action potential which was not recorded). Records retouched.

the end-plate potential at the skeletal neuromuscular junction in presence of high Mg^{2+} concentration (del Castillo & Katz, 1954*a*, *b*). This suggests that the 'quantal content' (i.e. the number of quanta released by a single stimulus) of the normal synaptic potential is low: Sacchi & Perri (1971)

have found that the quantal content of synaptic potentials in the superior cervical ganglion of guinea-pigs was approximately 1·5.

Minimal synaptic potentials lasted for from 25 to 60 msec. As it was not possible to measure their duration with any accuracy it was decided to measure the time from half-rise to half-decay (subsequently referred to as half-width) (Jack & Redman, 1971*a*, *b*). As the distribution of the half-widths of synaptic potentials for any one cell is roughly Gaussian (e.g. Fig. 3) it is reasonable to calculate the mean half-width of the synaptic potentials recorded from a given cell. The means for thirty-three control cells are shown in the histogram of Fig. 2.

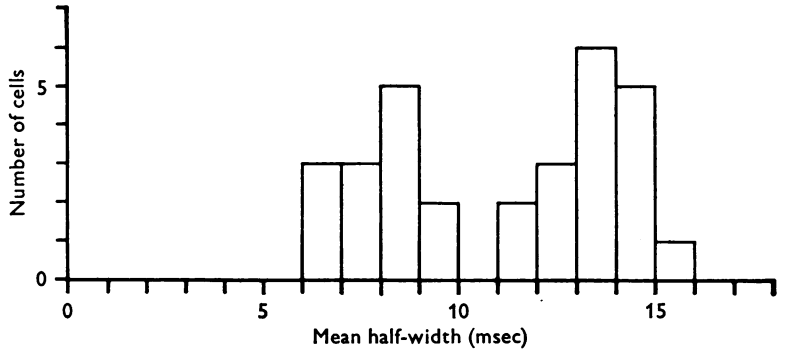

Fig. 2. Histogram of the distribution of mean half-widths of synaptic potentials recorded from thirty-three cells.

There appeared to be two groups of synaptic potentials, one group with half-widths from 6·1 to 9·5 msec and a second ranging from 11·5 to 15·8 msec. The difference between the means of the two apparent distributions was tested statistically using the Student *t* test and was found to be significant ($P \ll 0{\cdot}001$). As these groups are made up of mean half-widths from different cells, the possibility arises that records have been obtained from two different types of cell. The distribution of thresholds was examined for a number of cells identified as having a given type of synaptic potential; the distributions were such that cells with long synaptic potentials had significantly lower thresholds than those with shorter synaptic potentials ($P \ll 0{\cdot}001$). This suggests that there are two types of ganglion cell in the inf. mes. gang. of guinea-pigs (see Crowcroft & Szurszewski, 1971). The type of cell with short synaptic potentials and high (mean 29·5 mV) thresholds will be referred to as type S and those with long synaptic potentials and low (mean 14·6 mV) thresholds will be termed type L.

Rise times (i.e. the time from base line to peak) of the synaptic potentials

varied from 2·2 to 5·6 msec in type S cells and from 3·6 to 7·1 msec in type L cells. The distributions of rise times for the two different types of cell were found to be significantly different ($P \ll 0{\cdot}001$).

Effect of physostigmine on the time course of synaptic potentials

In some cells it was possible to examine synaptic potentials before and after exposure to physostigmine ($1{\cdot}2 \times 10^{-6}$ M). In all such cells, the half-

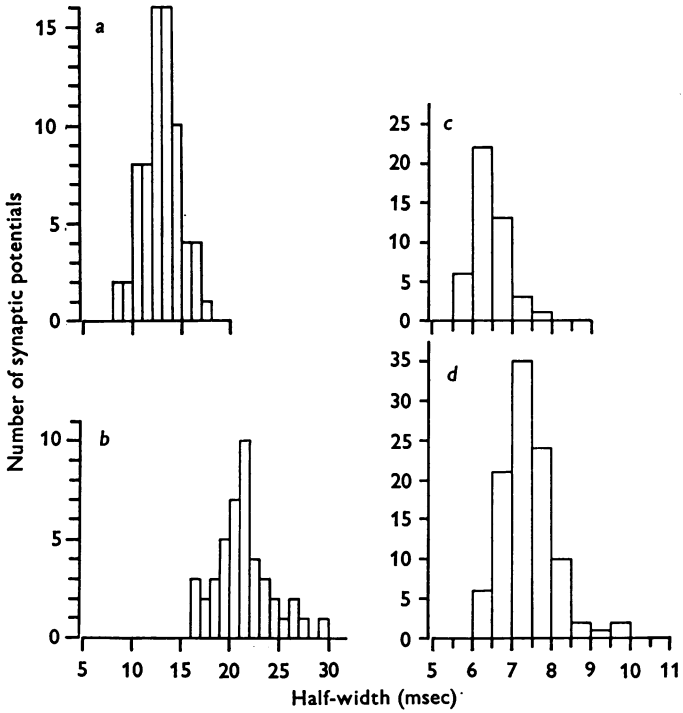

Fig. 3. Histograms showing the distribution of half-widths for (*a*) seventy-two synaptic potentials from a type L cell, (*b*) forty-one synaptic potentials from the same cell after treatment with physostigmine, (*c*) forty-four synaptic potentials from a type S cell, (*d*) 100 synaptic potentials from the same cell after treatment with physostigmine (note the scales in *a* and *b* are different from those in *c* and *d*, as is the basal interval).

width of the synaptic potential was prolonged after treatment. In Fig. 3, distributions of half-widths from a type L cell (Fig. 3*a*, *b*) and a type S cell (Fig. 3*c*, *d*) (illustrated with records in Fig. 4), have been shown for before and after treatment with physostigmine. In each case there is an obvious change in half-width and these changes were found to be significant (type L cell $P \ll 0{\cdot}001$, type S cell $P < 0{\cdot}001$).

Most cells could not be held for more than 25 min, and this was insufficient time to collect data before and after adding physostigmine. To avoid this difficulty half-widths of 1115 synaptic potentials from thirty-three cells were compared with the half-widths of 1000 synaptic potentials from thirty-two cells taken at least 15 min after the application of physostigmine. These distributions are shown in Fig. 5. The control distribution in Fig. 5 appears to be bimodal, reflecting the two populations of cells already described. The distribution of values obtained in the presence of physostigmine shows marked changes; the rising phase is shifted, there is a tailing out to longer durations and there appears to be only one peak.

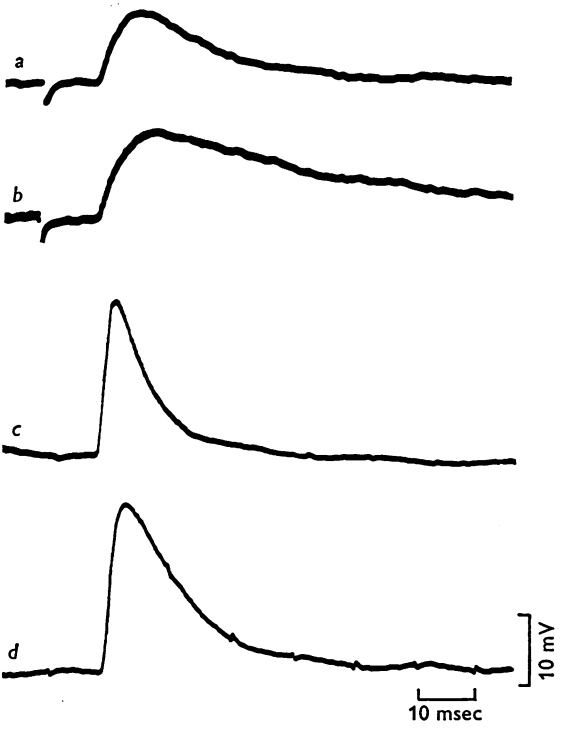

Fig. 4. Typical synaptic potentials from (*a*) a type L cell (that of Fig. 3 *a* and *b*), (*b*) the same cell after treatment with physostigmine, (*c*) a type S cell (that of Fig. 3 *c* and *d*), (*d*) the same cell after treatment with physostigmine (rise phases retouched).

To determine whether prolongation always occurs, the distribution of mean half-widths from each control cell was compared with the distribution of mean half-widths from cells treated with physostigmine. Fig. 6 shows these two distributions. Fig. 6*a* is a histogram of the mean half-

widths from thirty-three control cells (it is Fig. 2 replotted with a basal interval of 2 msec rather than 1 msec), while Fig. 6*b* is a histogram of mean half-widths from thirty-two cells, which were treated with physostigmine. There is a significant ($P \ll 0{\cdot}001$) shift in the distributions of treated half-widths, again indicating that the synaptic potentials of both type L and type S cells are prolonged by physostigmine.

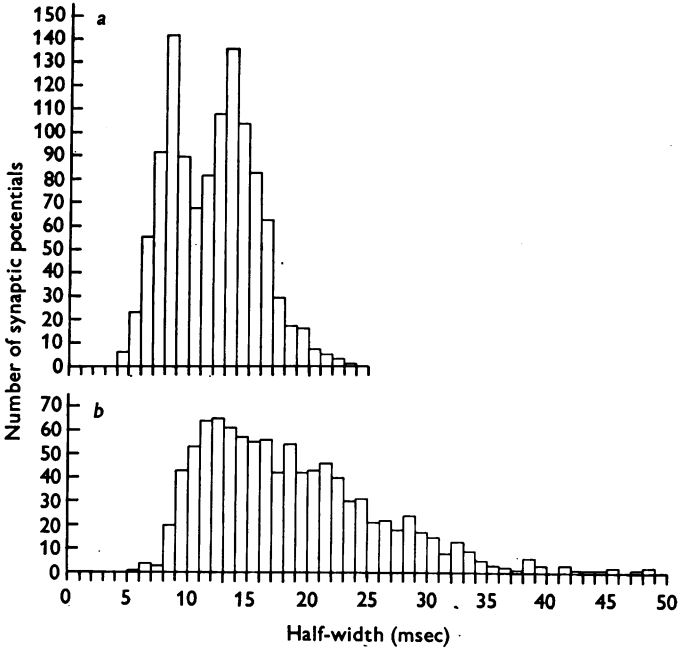

Fig. 5. Histograms showing the distribution of half-widths for (*a*) 1115 synaptic potentials from thirty-three control cells, and (*b*) 1000 synaptic potentials from thirty-two cells after the application of physostigmine.

Similar histograms were plotted for rise times (Fig. 7) and these showed that rise times were prolonged by physostigmine ($P \ll 0{\cdot}001$).

In all ganglion cells, physostigmine caused threshold (the change in membrane potential at which an action potential was initiated) to be lowered by a variable amount, usually about 8 mV. If a synaptic potential was just subthreshold then it might be prolonged by an active response of the membrane. To test this possible effect on the results obtained in the presence of physostigmine, synaptic potentials were recorded while a hyperpolarizing current pulse was applied through the recording electrode. In most cells this did not affect the half-width of the synaptic potential after physostigmine; but in some cells the half-width was reduced, although it was still longer than that of the control.

Spontaneous synaptic potentials

Spontaneous synaptic potentials were seen in almost every cell. Their frequency was very low, usually less than 1/min. Repetitive stimulation has been reported by Blackman *et al.* (1969) to increase the frequency of spontaneous synaptic potentials in the hypogastric ganglia. The ascending mesenteric nerve was stimulated at 20–30 Hz for varying periods in an attempt to obtain enough data for analysis. In most cells this was ineffective, and the frequency of spontaneous synaptic potentials was not noticeably increased.

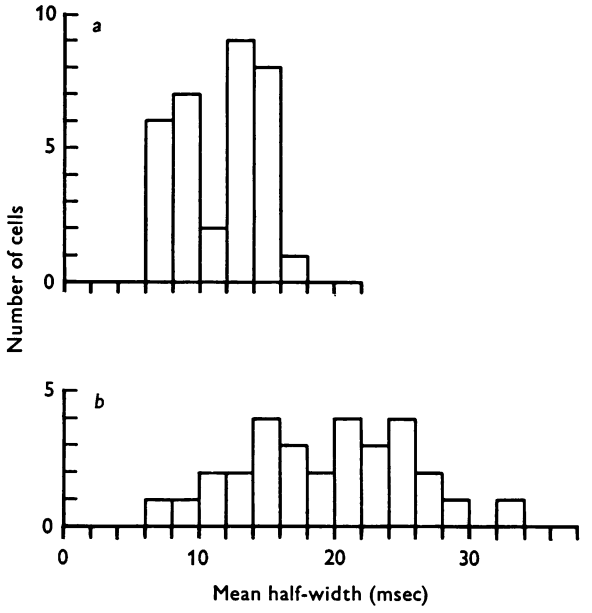

Fig. 6. Histograms showing the distribution of mean half-width for (*a*) thirty-three control cells; and (*b*) thirty-two cells after treatment with physostigmine.

In four cells, the number of spontaneous synaptic potentials recorded before and after treatment with physostigmine was sufficient to give a reasonable sample for statistical analysis. As shown in Table 1, a significant increase in half-width was observed.

Although physostigmine caused some depolarization, as indicated by the fall in threshold, a marked increase in the amplitude of the smallest spontaneous synaptic potentials was observed. This may be assumed to reflect an increase in the amplitude of the potential change produced by one quantum of ACh.

Other effects of physostigmine

Repetitive stimulation of the ascending mesenteric nerve, in the presence of physostigmine, led to the spontaneous firing of action potentials (as reported by Eccles, 1944). This response could be blocked by addition of atropine ($1{\cdot}4 \times 10^{-7}$ M) to the bathing medium. Fig. 8 shows the responses to repetitive stimulation at 20 Hz for (i) 11 sec in a control cell,

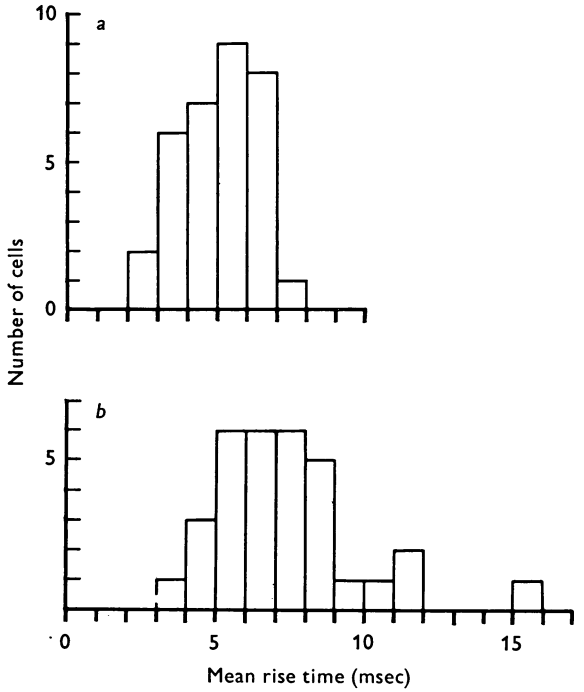

Fig. 7. Histograms showing the distribution of mean rise times for the synaptic potentials for (*a*) thirty-three control cells; and (*b*) thirty-two cells after treatment with physostigmine.

TABLE 1. The durations of miniatures from four cells before and after treatment with eserine

	Control			After eserine		
Cell type	No. miniatures	Duration (msec)	Amplitude (mV)	No. miniatures	Duration (msec)	Amplitude (mV)
L	39	13·4	1·5	33	21·6	2·5
L	61	13·5	1·0	25	27·8	2·5
S	21	4·74	0·5	10	7·25	1·0
L	30	12·02	1·5	6	24·0	2·5

(ii) 3·9 sec in a cell treated with physostigmine and (iii) 20 sec in the same cell treated with physostigmine and atropine. In the control experiment the stimulus strength was suprathreshold (i.e. a single volley always gave an action potential as several presynaptic fibres were stimulated). There was a burst of spontaneous synaptic potentials at the end of the train of stimuli. After physostigmine (8 ii) a shorter period of minimal stimulation (a single stimulus gave only a single synaptic potential) led to rhythmic firing of action potentials by the end of the period of stimulation; 1 min

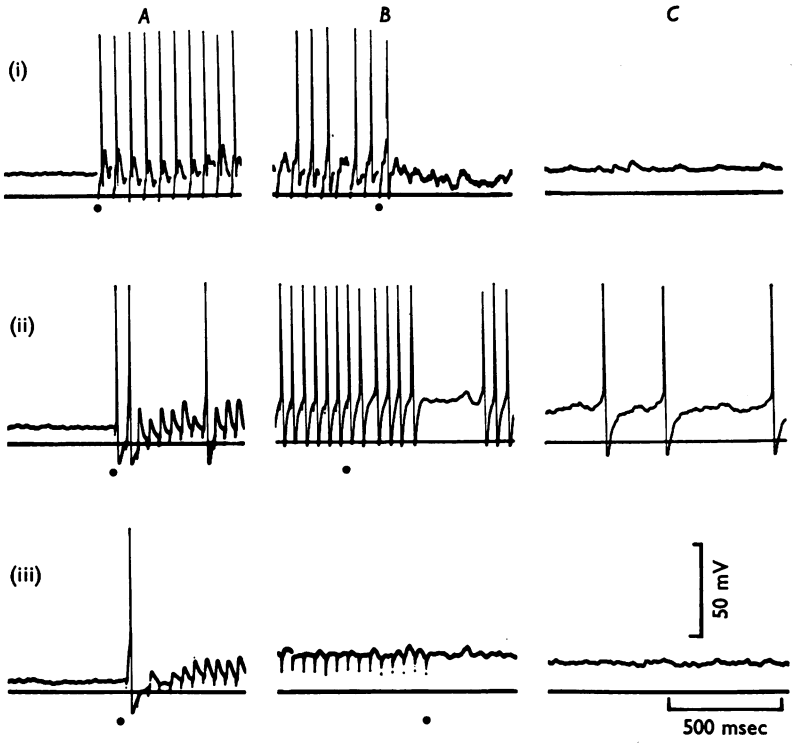

Fig. 8. Effects of repetitive stimulation at 20 Hz; records in column *A* taken at the beginning of the period of stimulation, *B*, at the end of the train of stimuli, *C*, 1 min later. Row (i), control cell; row (ii), after treatment with physostigmine; row (iii), after treatment with physostigmine and atropine. See text for explanation (records retouched).

after the stimulation ceased spontaneous action potentials could still be observed. Atropine (8 iii) blocked this response despite a much longer period of stimulation. These results are typical of those obtained after repetitive stimulation for type L cells. Some type S cells did not show spontaneous action potentials.

In physostigmine-treated cells spontaneous firing often continued up to 30 min after a burst of stimuli. The addition of atropine completely blocked this effect. To determine the mechanism underlying the spontaneous firing of action potentials, long hyperpolarizing current pulses were passed through the recording electrode during a period of spontaneous activity. This failed to reveal any changes in membrane potential which might be responsible for the spontaneous action potentials (see Fig. 9).

In control cells a long depolarizing current pulse mimicked the spontaneous firing seen in a physostigmine-treated cell (see Fig. 10). After blocking the spontaneous firing with atropine a depolarizing current pulse

Fig. 9. The effect of hyperpolarizing a ganglion cell during spontaneous firing of action potentials (upper trace membrane potential, lower trace current). Traces *a* and *b* are separated by a 20 sec period of maintained hyperpolarization.

could again mimic the pattern of spontaneous activity. As atropine also increases threshold in physostigmine-treated cells, presumably by causing repolarization of the membrane, the spontaneous firing could be due to a long-lasting depolarization of the cell membrane caused by persistent action of ACh on muscarinic receptors.

It was observed that during prolonged periods of presynaptic stimulation the amplitude of synaptic potentials recorded from physostigmine-treated cells declined. This was most obvious in cells where spontaneous firing was not observed or was blocked by atropine. The decline in amplitude of synaptic potentials is illustrated in Fig. 8(iii) where, by the end of the stimulation period (in the presence of physostigmine and atropine), the amplitude of the synaptic potentials had markedly declined. The differences in amplitude between successive synaptic potentials at the beginning and end of the period of stimulation are of the same magnitude

and appear to be 'quantal' in nature; thus, it is unlikely that receptor desensitization is responsible for the observed decline in amplitude. ACh synthesis relies on choline produced by ACh hydrolysis; physostigmine by blocking cholinesterase will lead to a decline of ACh synthesis, and thus cause a decline in the amplitude of synaptic potentials during long periods of stimulation (see Bennett & McLachlan, 1972*a*, *b*).

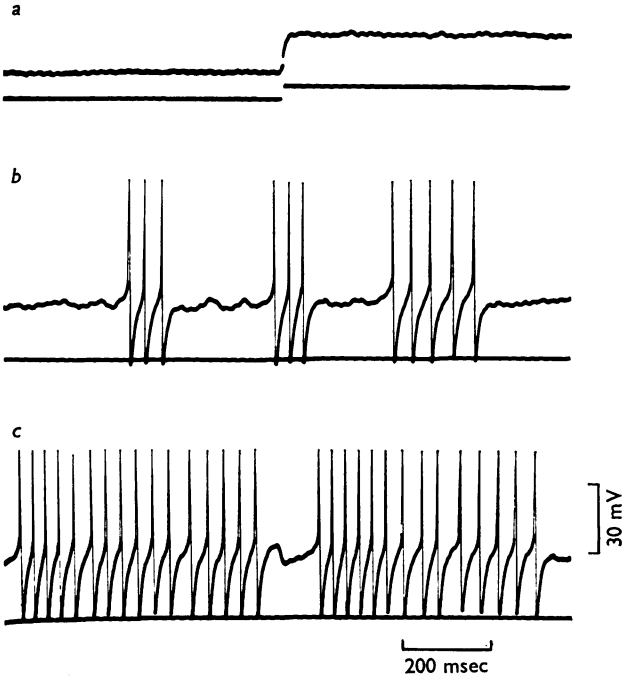

Fig. 10. Control cell showing the effects of prolonged depolarization. All records from the same cell. The values of the depolarizing currents were $3{\cdot}5 \times 10^{-10}$ A (*a*), $4{\cdot}0 \times 10^{-10}$ A (*b*) and $4{\cdot}2 \times 10^{-10}$ A (*c*).

As atropine blocked some of the effects of physostigmine (i.e. the reduction of threshold and the spontaneous action potentials following repetitive stimulation), it was thought that atropine might block the prolongation of the synaptic potentials seen after treatment with physostigmine. The effect of atropine upon the duration of synaptic potentials was examined before and after treatment with physostigmine; it was found that atropine did not affect the normal synaptic potential nor did it block the prolongation due to physostigmine. Atropine did shorten synaptic potentials in some physostigmine-treated cells, but these potentials were still prolonged. In other cells atropine had no effect.

DISCUSSION

These experiments have shown that physostigmine causes a prolongation of the half-width and rise times of minimal synaptic potentials recorded from a mammalian ganglion. Inhibition of AChE also causes a prolongation of the half-width of spontaneous synaptic potentials which have been assumed by previous workers to be due to the release of a quantum of ACh (Blackman *et al.* 1963*b*). It must be emphasized that although these effects were statistically significant the action of physostigmine at $1{\cdot}2 \times 10^{-6}$ M was far less dramatic than that which is observed following inhibition of AChE at the skeletal neuromuscular junction, where miniature end-plate potentials are prolonged far more than could be explained by simple diffusion (Katz & Miledi, 1973). Ogston (1955) and Eccles & Jaeger (1957) have calculated that diffusion of ACh away from the synapse in ganglia is sufficiently fast to account for the time course of the normal synaptic potential; however, if the 'trapping' effect described by Katz & Miledi (1973) is included a longer diffusion time would be obtained. The duration of the synaptic potentials seen after physostigmine treatment might be explained by this longer diffusion time.

Two types of cell were observed in the imf. mes. gang. of guinea-pigs. Type L cells had long synaptic potentials and low thresholds, while type S cells had short synaptic potentials and high thresholds. These might be correlated with the ganglion cell types described in the inf. mes. gang. by Crowcroft & Szurszewski (1971) as type I cells (low membrane potentials, low thresholds and high input resistances) and type II cells (high membrane potentials, high thresholds and low input resistances). Inhibition of AChE causes a significant prolongation of synaptic potentials in both these types of cell.

Previous studies of the effects of anticholinesterases on mammalian sympathetic ganglia have been made using surface electrodes or more indirect recording techniques, e.g. measuring contractions of the nictitating membrane as an index of activation of the superior cervical ganglion (Feldberg & Vartiainen, 1934*b*; Eccles, 1934, 1935, 1937, 1944; Rosenblueth & Simeone, 1938; McIsaac & Koelle, 1959; Volle & Koelle, 1961; Gillis, Flack, Garfield & Alper, 1968; Kosterlitz, Lees & Wallis, 1968). Most of these authors found that transmission of a submaximal stimulus through the ganglion was potentiated by anticholinesterases, but they offered a number of different explanations for this result. The results described here suggest that potentiation might be due to two processes.

1. The prolongation of the duration of synaptic potentials caused by the action of ACh on nicotinic receptors. As mentioned earlier, in the guinea-pig inf. mes. gang. and in many other mammalian ganglia sum-

mation is necessary in order to excite the cell (see Skok, 1973). A relatively small prolongation of the duration of the synaptic potential could be effective in facilitating transmission if this was assessed in terms of the total post-synaptic discharge (i.e. the complex action potential recorded extracellularly from post-ganglionic nerves).

2. The accumulation of ACh within the interstices of the ganglion and its continuing action on muscarinic receptors. This action of ACh appears to be depolarization of a magnitude sufficient to bring the membrane potential of many ganglion cells into a zone where the cell tends to fire repetitively. The exact magnitude of the depolarization could not be determined in the present experiments, and the possibility that muscarinic receptors may alter the excitability of ganglion cells by mechanisms other than a change in resting membrane potential cannot be ruled out.

The time course of a synaptic potential is determined by two factors: the time course of the synaptic current and the passive electrical properties of the membrane. If physostigmine caused an increase in membrane resistance synaptic potentials would be prolonged. The muscarinic action of ACh in frog sympathetic ganglia is to cause depolarization by an increase in cell input resistance due to a reduction in the permeability of the membrane to K^+ (Nishi, 1970; Kobayashi & Libet, 1970). However Kobayashi & Libet (1970) found that in the rabbit superior cervical ganglion the muscarinic depolarization was not associated with a significant increase in cell input resistance. In the present experiments atropine did not abolish the effect of physostigmine in prolonging the synaptic potentials so that, even if some increase in membrane resistance had occurred it seems unlikely that this was of sufficient magnitude to explain the action of physostigmine. Thus the simplest explanation would appear to be a prolongation of the time during which ACh molecules were in the vicinity of nicotinic receptors.

Eccles (1944) observed that repetitive preganglionic stimulation led to a depolarization, which persisted after the stimulation ceased; this could occasionally produce a brief burst of spontaneous action potentials. In the presence of physostigmine this behaviour was potentiated so that spontaneous firing persisted for up to 10 sec. This finding has been confirmed by several workers and it was shown that the depolarization was due to the action of ACh on muscarinic receptors (Takeshige & Volle, 1963; Volle, 1962; Volle & Koelle, 1961). The prolonged time course of repetitive firing following the release of ACh by repetitive stimulation (up to 30 min) is of interest. However, the present experiments were carried out on the inf. mes. gang. *in vitro* and it is possible that ACh may remain trapped within the interstices of the ganglion and that this could account for this phenomenon.

No evidence was obtained for the hypothesis that the accumulation of ACh might cause the release of ACh from presynaptic terminals; i.e. when spontaneous firing was blocked by hyperpolarizing the cell membrane, there was no observable increase in the frequency of spontaneous synaptic potentials although they were prolonged in the same way as evoked potentials.

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