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Impulse transmission in the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum

H. W. KOSTERLITZ AND R. J. LYDON

Department of Pharmacology, University of Aberdeen

Summary

1. In a preparation consisting of the myenteric plexus and the longitudinal muscle layer removed from a segment of guinea-pig ileum, spontaneous action potentials occurred which were unaffected by tetrodotoxin but suppressed by Mn^{2+} and were therefore myogenic.

2. A single current pulse of 0·1 ms duration evoked a response consisting of an early action potential followed after a delay of about 200 ms by a complex of biphasic spikes. The first action potential was conducted for no more than 15 mm and the second complex for 30–70 mm.

3. Since the first action potential was unaffected by hyoscine or Mn^{2+} but abolished by tetrodotoxin, it was due to excitation of nerve fibres. The later complex of spikes was suppressed by hyoscine and Mn^{2+} and therefore due to excitation of smooth muscle. It was also inhibited by adrenaline or morphine, compounds which depress acetylcholine release. The evoked smooth muscle response was followed by absence of spontaneous electrical activity for 2–4 seconds.

4. The nerves travelling in a longitudinal direction had a mean maximum conduction velocity of 0·65 m/s, an absolute refractory period of 2·8 ms and a relative refractory period of about 20 ms.

5. The conduction velocity of the smooth muscle response evoked by stimulation of the nerve with a single pulse was 0·16 m/second. After a single pulse the muscle was inexcitable for 0·7–1·3 s; the delay of transmission from nerve to muscle was 210 ms. When instead of a single pulse a train of two–five pulses at 20 ms intervals was applied, the size, conduction distance and conduction velocity of the evoked smooth muscle response were increased.

Introduction

The myenteric plexus-longitudinal preparation was first introduced by Ambache (1954), modified by Rang (1964) and by Kosterlitz, Lydon & Watt (1970). It was used for studies of the release of acetylcholine by Cowie, Kosterlitz & Watt (1968), Vizi (1968), Paton & Zar (1968), Paton & Vizi (1969) and Kosterlitz *et al.* (1970).

In view of the interest in this preparation, an electrophysiological analysis of the mechanism of transmission from the plexus to the smooth muscle appeared to be important. A preliminary report of some of the findings was presented to the Physiological Society (Kosterlitz & Lydon, 1969).

Methods

Experimental procedures

Male and female guinea-pigs, 300–900 g, were killed by a blow on the back of the neck and bled from the carotid arteries. A suitable length of ileum was removed and placed in warm Krebs solution. The terminal 10 cm of ileum, which has excitatory α-adrenoceptors (Munro, 1953), was avoided. The method of removing the longitudinal muscle with the adherent myenteric plexus, based on the techniques of Ambache (1954) and Rang (1964), has already been described (Kosterlitz *et al.*, 1970).

The experiments were carried out in a moist chamber similar to that described by Eccles (1952) for recording action potentials in isolated mammalian ganglia and used for the same purpose by Kosterlitz & Wallis (1966). The plexus-longitudinal muscle preparation tends to curl into a cylindrical shape with the muscle outermost. A 6 cm length was tied with nylon thread to Perspex posts coming down from the removable top of the moist chamber. The electrodes were of platinum wire of 0·3 mm diameter and were also mounted in the top of the moist chamber. Pairs of electrodes, one pair being movable, were used for stimulation and recording. Each pair of electrodes consisted of two wires 3 mm apart, arranged transversely on the muscle strip. The preparation was earthed through a single platinum wire placed between the two pairs.

At least 1 min before stimulating and recording, the chamber was tilted to raise the preparation out of the Krebs solution (50 ml at 35–36° C). Stimulation was with rectangular pulses from a Grass S8 stimulator and isolation unit. The stimuli were applied every 2 s unless stated otherwise and consisted of one–five pulses spaced at intervals of 20 ms; that is, the stimulus frequency was 0·5 Hz and the pulse frequency 50 Hz. The pulses were of supramaximal voltage and 0·1 ms duration. The action potentials were led off by means of cathode followers into a capacity-coupled amplifier and displayed on an oscilloscope and on a pen oscillograph. Time constants of 0·02 or 0·2 s were chosen to eliminate the slow waves. Mean values are given with their standard errors.

Solution and drugs

The Krebs solution had the following composition (mM): NaCl, 118; KCl, 4·75; $CaCl_2$, 2·54; KH_2PO_4, 1·19; $MgSO_4$, 1·19; $NaHCO_3$, 25; and glucose, 11; it was bubbled with 95% O_2 and 5% CO_2. Mepyramine (125 nM) was included in the Krebs solution to antagonize the action of any histamine that might be released by the preparation.

The following drugs were used: acetylcholine chloride, adrenaline, ascorbic acid, hyoscine hydrobromide, mepyramine maleate, morphine hydrochloride and tetrodotoxin. For stock solutions, acetylcholine was made up in 5% NaH_2PO_4 solution, adrenaline in 0·01 N HCl, and the other drugs in distilled water. The final dilution of adrenaline, of which 0·1 ml was injected into the chamber, contained ascorbic acid (1·13 mM). In preliminary experiments ascorbic acid, 0·113 mM, had no effect on electrical activity; therefore, in all experiments with adrenaline, the Krebs solution contained this concentration of ascorbic acid. $MnCl_2.4H_2O$ or $MnSO_4.4H_2O$ served as source of Mn^{2+}. The final concentrations of drugs and salts are expressed as mM or μM of the active component.

Results

Spontaneous activity

When the preparation was not stimulated electrically, it exhibited spontaneous electrical activity characteristic of intestinal smooth muscle. This activity was seen as spindle-shaped bursts of spikes alternating with more continuous discharge (Fig. 1). When displayed on an oscilloscope, the spontaneous activity consisted of biphasic action potentials of varying amplitude and duration. Neither hyoscine nor tetrodotoxin had any effect on the spontaneous discharge of action potentials, though they were abolished by Mn^{2+} (1 mM, Fig. 1C).

Excitatory response to electrical stimulation

When a stimulus consisting of a single pulse was applied to the preparation and the evoked response recorded at a distance of 5–10 mm, the beginning of the response was often somewhat obscured by spontaneous activity. This difficulty was overcome by making use of the fact that the response was followed by a period of quiescence lasting for 2–4 s (Fig. 2). If stimuli were applied at a frequency of 0·5 Hz so that each arrived during the quiescent period following the previous response, the nature of the response was clearly seen (Fig. 3A). There were two distinct components to the evoked response: an early compound action potential, seen better at a faster sweep speed, followed after a delay of about 200 ms by an irregular complex of spikes. Mn^{2+} (1 mM) abolished the second complex component of the response while leaving the first component unaffected (Fig. 3B);

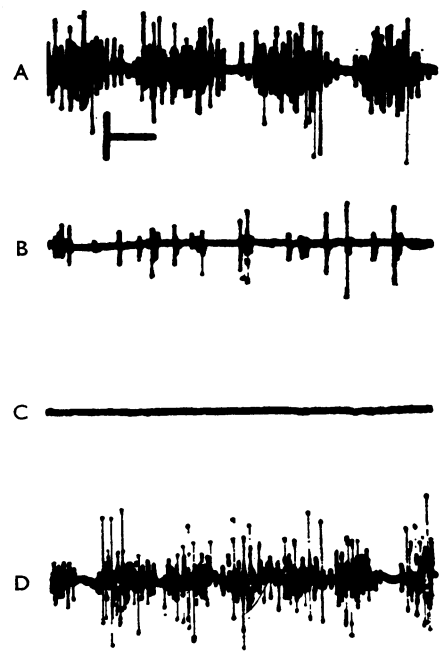

FIG. 1. Effect of Mn^{2+} on the spontaneous electrical activity of the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum. A, Control; B, in the presence of 0·5 mM Mn^{2+}; C, in the presence of 1 mM Mn^{2+}; D, after washing out Mn^{2+}. Calibrations: 50 μV, 5 seconds.

when tetrodotoxin (0·1 μg/ml) was then added to the Krebs solution in addition to the Mn^{2+}, the first component was also abolished (Fig. 3C) leaving only the stimulus artifact. When tetrodotoxin (0·1 μg/ml) was added without Mn^{2+} both components of the evoked response were abolished and spontaneous activity was seen in their place.

Drugs which affect transmission from nerve to muscle prejunctionally, for example adrenaline (0·25 μM) or postjunctionally, for example hyoscine (0·2 μM), abolished the second component of the evoked response without affecting the first component (Fig. 4). Morphine (0·07 μM) had a similar effect (Fig. 5).

Evoked nerve action potential

The determination of the chronaxie for the first component of the evoked response produced further evidence that this component was an action potential resulting from electrical excitation of the nerves. From strength-duration curves the chronaxie was calculated to be of the order of 0·1 ms (Fig. 6). Absolute and refractory periods were determined by measuring the current required to produce a threshold response in the nerves at various time intervals after a supramaximal conditioning stimulus. In Fig. 7, a typical result is shown as a plot of relative

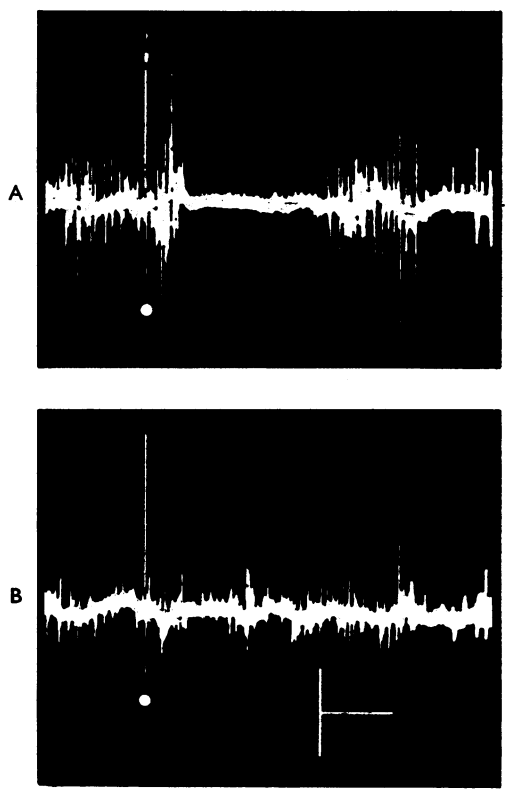

FIG. 2. Quiescent period following an evoked smooth muscle action potential (A) and the suppression of the action potential and absence of the quiescent period in the presence of hyoscine (0·2 μM, B). Single pulses (0·1 Hz) applied at dots; large nerve action potential seen in both tracings. Calibrations: 50 μV, 1 second.

excitability of the fibres recovering most rapidly against the interval between conditioning and test stimuli. The mean absolute refractory period was $2{\cdot}8 \pm 0{\cdot}2$ ms ($n=6$) and the relative refractory period about 20 ms.

The evoked nerve action potential diminished rapidly in size as the conduction distance was increased (Fig. 8) and could never be recorded when the distance from the stimulating cathode to the recording electrodes was greater than 15 mm. It could be recorded equally well in an oral or aboral direction ; hexamethonium (140 μM) had no effect on the action potential.

When the conduction time was plotted against the distance from the stimulating cathode to the first of the recording electrodes, a straight line was obtained which passed through the origin (Fig. 9). The conduction velocity of the fastest fibres at 35–36° C was $0{\cdot}65 \pm 0{\cdot}02$ m/s ($n=15$).

Evoked smooth muscle action potential complex

When the distance between the stimulating and the recording electrodes was changed, the shape and size of the evoked smooth muscle response varied, sometimes considerably. This was so whether the recording or stimulating electrodes

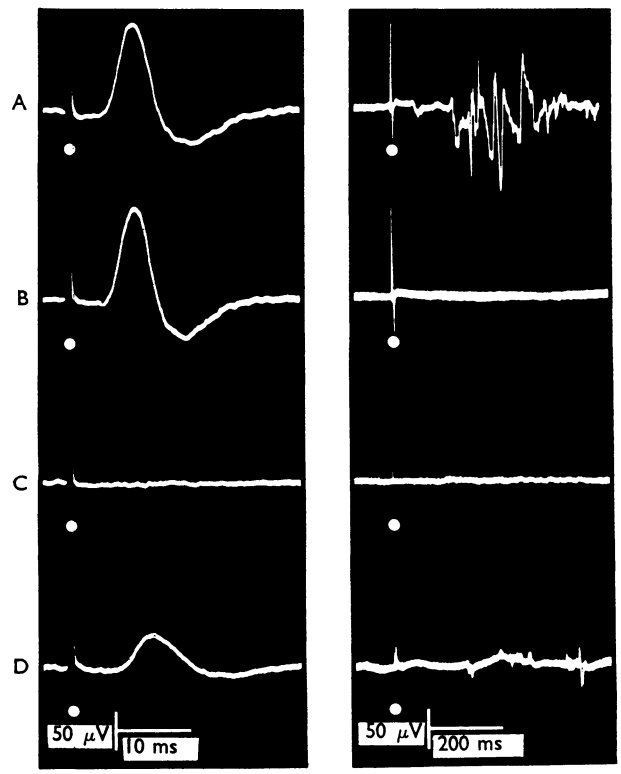

FIG. 3. Effects of Mn^{2+} and tetrodotoxin on the evoked nerve and smooth muscle action potentials. Sweep speed of left hand tracing faster than that of right hand tracing. A, Controls ; B, in the presence of Mn^{2+} (1 mM) ; C, in the presence of Mn^{2+} (1 mM) and tetrodotoxin (0·1 μg/ml) ; D, after washing out Mn^{2+} and tetrodotoxin. Single pulses at a frequency of 0·5 Hz applied at dots. Calibrations: 10 ms (left), 200 ms (right), 50 μv (both sides).

were moved. The smooth muscle conducted in either direction from the point of stimulation of the intrinsic nerves. The distance over which different preparations conducted varied; some preparations conducted for no more than 20–30 mm, while others conducted along their whole length of 60–70 mm.

When the applied stimulus consisted of a single pulse, 0·1 ms in duration and of a voltage 20% higher than that required for a maximal nerve action potential, the smooth muscle required an interval of 0·7–1·3 s between stimuli before it would conduct a response every time. When the interval between stimuli varied between 1·4 and 10 s, the latency between stimulus artifact and the beginning of the smooth muscle action potential decreased with increasing interval.

Conduction velocity was determined by measuring the latency of the evoked smooth muscle action potential at a frequency of 0·5 Hz. As already mentioned, spontaneous electrical activity is thus suppressed during the period of the evoked response. The mean conduction velocity was $0{\cdot}16 \pm 0{\cdot}02$ m/s ($n=9$). The results of a typical experiment are shown in Fig. 10. By extrapolation to zero distance of the line obtained by plotting latency against conduction distance, an approximate measure of the mean apparent junctional delay was obtained, namely 210 ± 13 ms ($n=9$).

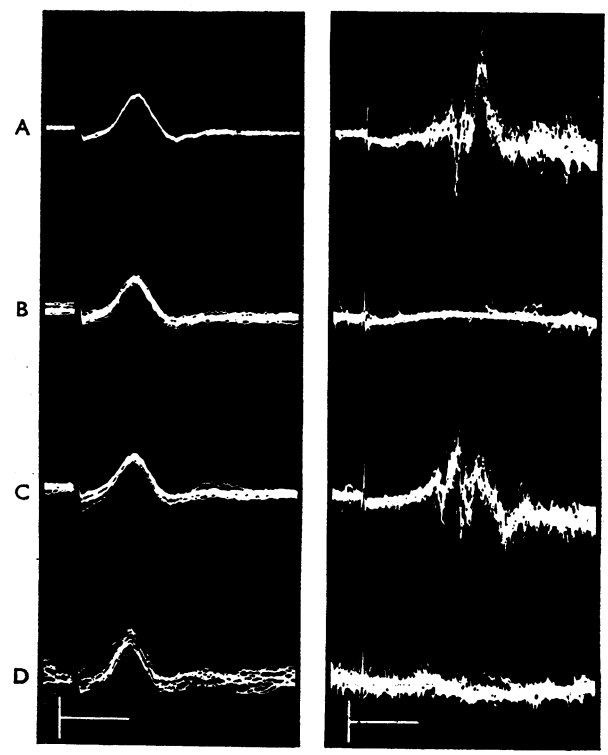

FIG. 4. Effects of adrenaline and hyoscine on the evoked smooth muscle action potentials. Sweep speed of left-hand tracing faster than that of right-hand tracing. Ten sweeps are superimposed in each case. A, Controls; B, in the presence of adrenaline (0·25 μM); C, after washing out adrenaline; D, in the presence of hyoscine (0·2 μM). Stimulation by single pulses at a frequency of 0·5 Hz. Calibrations: 10 ms (left), 200 ms (right), 50 μV (both sides).

Although the electrical stimulus applied to the nerves in these experiments was supramaximal for the nerves, the response of the smooth muscle was increased when, instead of a single pulse, trains of two–five pulses with intervals of 20 ms between each pulse were used as stimulus. It was found that the size of the smooth muscle complex increased, the latency of the smooth muscle response

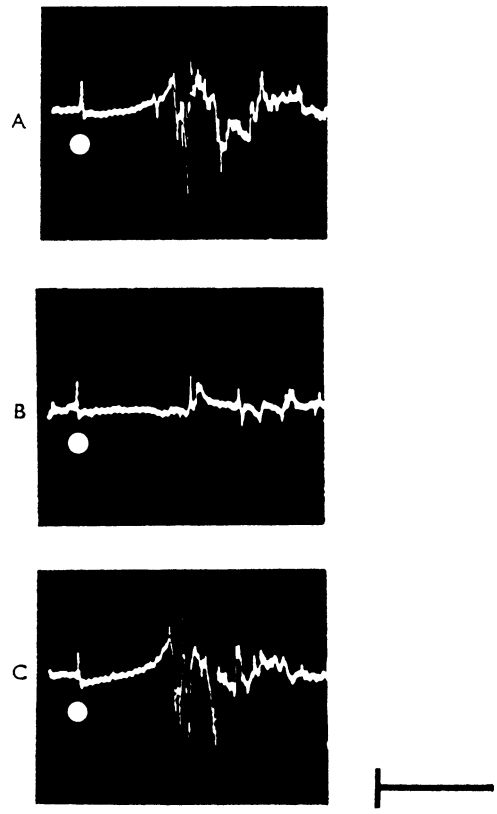

FIG. 5. Effect of morphine on the evoked smooth muscle response. A, Control; B, in the presence of morphine (0·07 μM); C, after washing out morphine. Single pulses at a frequency of 0·5 Hz applied at dots. Calibrations: 500 ms, 50 μV.

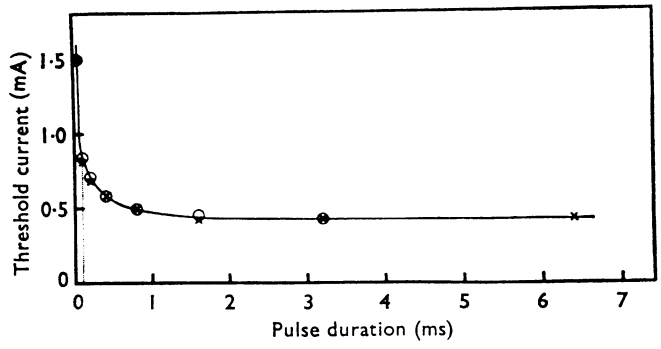

FIG. 6. Strength-duration relationship for the evoked nerve action potential. Abscissa, pulse duration (ms); ordinate, current required to evoke a threshold response (mA). Conduction distance: ○, 2·5 mm; ×, 5 mm. Chronaxie, 0·1 ms (vertical dotted line).

decreased (Fig. 11) and the maximum conduction distance became larger. For instance, conduction velocity increased from 0·10 to 0·46 m/s (Fig. 12). Since the extrapolated regression lines obtained for stimuli of one–five pulses intersected at zero conduction distance, there was no change in the apparent junctional delay.

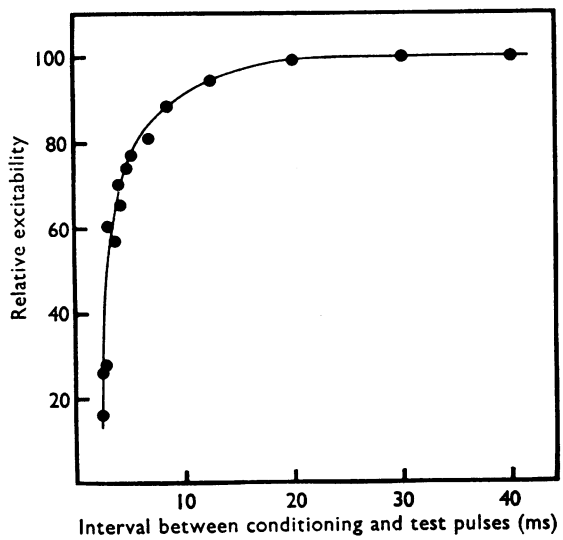

FIG. 7. Recovery of excitability of the nerves after a single supramaximal conditioning pulse. Abscissa, interval between conditioning and test pulses (ms); ordinate, relative excitability (threshold current of test pulse without conditioning pulse $\times 100$/threshold current of test pulse after conditioning pulse).

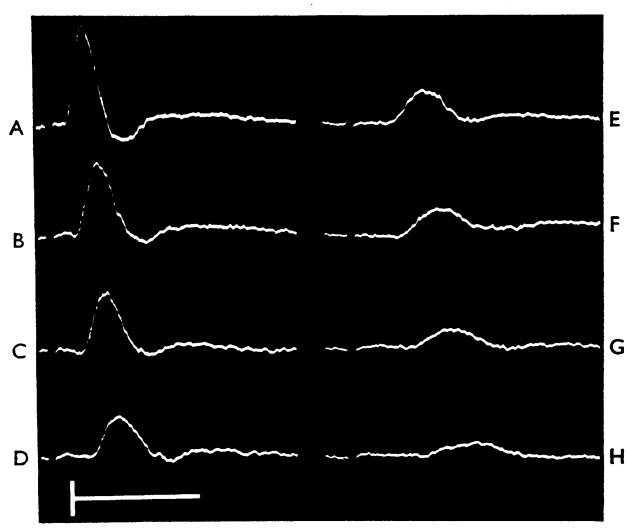

FIG. 8. Evoked nerve action potentials recorded at various distances from the stimulating cathode. The conduction distance of the first record (A) was 2 mm; each subsequent record was taken 1 mm further away from the stimulating cathode. Calibrations: 50 μV, 20 ms.

Discussion

The pharmacological evidence indicates that the spontaneous electrical activity is myogenic. As in other smooth muscle preparations, tetrodotoxin is without effect and Mn^{2+} abolishes activity (Kuriyama, Osa & Toida, 1966, 1967; Nonomura, Hotta & Ohashi, 1966; Hashimoto & Holman, 1967; Bülbring & Tomita, 1967). Since hyoscine has no inhibitory effect, release of acetylcholine is not necessary for spontaneous electrical activity.

The first component of the evoked action potential is assumed to be due to excitation of the fine nerve fibres of the myenteric plexus since the chronaxie is only 0·1 ms and the response is abolished by tetrodotoxin. This view is supported by the absence of any effects of Mn^{2+}, hyoscine, adrenaline and morphine. Moreover, Kuriyama, Osa & Toida (1967), showed that the smooth muscle cells of the

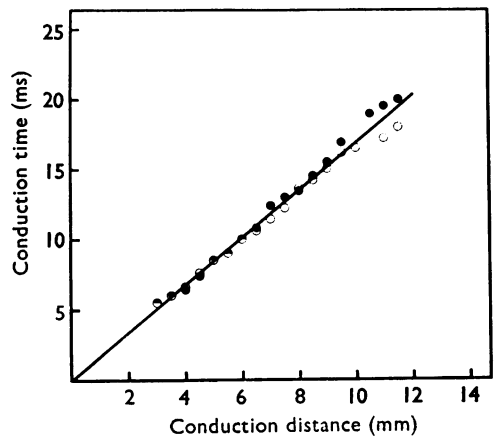

FIG. 9. Conduction velocity of the nerves. Abscissa, distance from stimulating cathode (conduction distance, mm); ordinate, time from the artifact to the beginning of the nerve action potential (conduction time, ms). ○, Recording electrode aborally from stimulating electrode; ●, electrodes in reverse order.

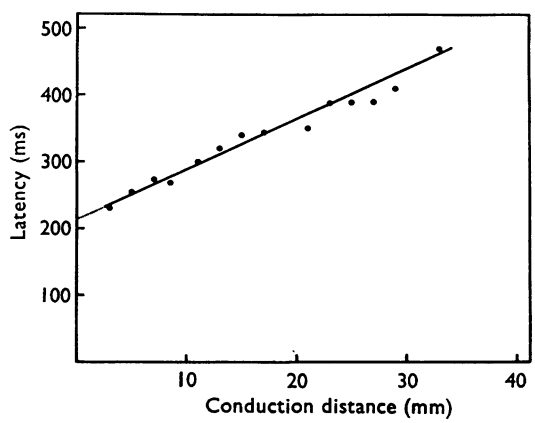

FIG. 10. Conduction velocity of the smooth muscle response evoked by single supramaximal pulses at a frequency of 0·5 Hz. Abscissa, distance from stimulating electrode (conduction distance, mm); ordinate, time from the artifact to the beginning of the smooth muscle action potential (latency, ms). The intercept with the ordinate is a measure for the nerve-muscle junctional delay.

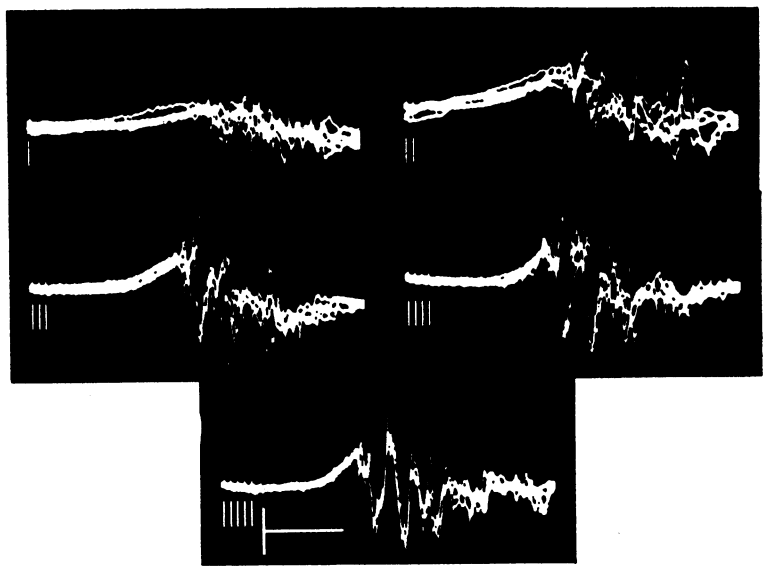

FIG. 11. Effects of an increase in the number of supramaximal pulses in a stimulating train on the evoked smooth muscle response. Intervals between trains, 2 s, and between pulses, 20 ms. Five responses are superimposed in each record. Conduction distance, 33 mm. The vertical white lines indicate the number of pulses in each train and the position of the artifacts. Calibrations: 50 μV, 200 ms.

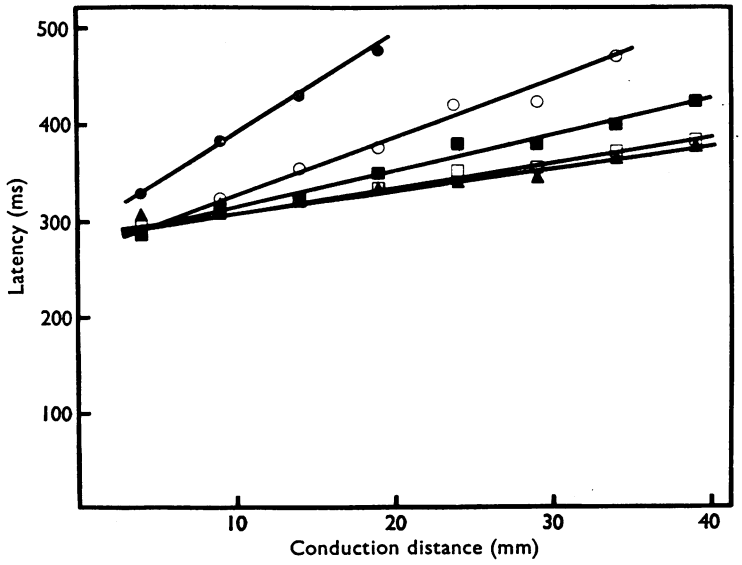

FIG. 12. Effects of an increase in the number of supramaximal pulses in a stimulating train on the conduction distance and conduction velocity of the smooth muscle action potential. Intervals between trains, 2 s, and between pulses, 20 ms. Abscissa, distance from stimulation cathode (conduction distance, mm); ordinate, time from the artifact to the beginning of the smooth muscle response (latency, ms). Number of pulses in stimulating trains: ●, one pulse (0·10 m/s); ○, two pulses (0·18 m/s); ■, three pulses (0·27 m/s); □, four pulses; ▲, five pulses (0·45 m/s). The last point on each line is the maximum conduction distance.

guinea-pig jejunum had chronaxies of 2–5 ms. The mean conduction velocity of the fastest fibres (0·65 m/s) and their absolute refractory period (2·8 ms) are similar to the values obtained for C fibres (Grundfest & Gasser, 1938).

It is not easy to say how far the nerves conduct along the preparation to excite the smooth muscle at points distant from the stimulating electrodes. Bülbring & Tomita (1967) deduced that, in the guinea-pig taenia, the intrinsic nerve fibres conduct for no more than a few millimetres in the longitudinal direction and it appears that the same is true for most of the excitatory nerves supplying the longitudinal muscle of the guinea-pig ileum. Moreover, on the evidence available at present it cannot be excluded that the evoked nerve action potential seen at distances greater than a few millimetres may be due to inhibitory non-adrenergic nerves (Kosterlitz & Lydon, 1969) that are travelling longitudinally.

The second component of the evoked response consists of a group of spikes. These are abolished by Mn^{2+}, which interferes with generation of muscle action potentials, by hyoscine, which causes postjunctional block, by morphine which causes prejunctional block (Paton, 1957, 1963 ; Cowie *et al.*, 1968 ; Paton & Zar, 1968) and by adrenaline which blocks mainly prejunctionally (Kosterlitz & Lydon, 1968 ; Vizi, 1968 ; Paton & Vizi, 1969 ; Kosterlitz *et al.*, 1970). These observations agree with the view that the second response is a complex of smooth muscle action potentials caused by the release of acetylcholine from the myenteric plexus.

The mean transmission delay of 210 ms after a single stimulus is of the same order as that found for the intramural excitatory nerves in the guinea-pig taenia (Bennett, 1966) and twice as long as that from the postsynaptic nerves to the longitudinal muscle of the rabbit colon (Gillespie, 1964). In this context, it is of interest that latency increases as stimulus frequency is raised from 0·1 to 0·7 Hz. The amount of acetylcholine released from the myenteric plexus by a single pulse is greater at low than at high frequencies (Paton, 1957, 1963 ; Cowie *et al.*, 1968 ; Paton & Zar, 1968).

The conduction velocity of the smooth muscle after the excitation of nervous structures by a single pulse was 0·16 m/second. This value is higher than the values obtained by direct stimulation of smooth muscle ; for instance, the conduction velocity in the longitudinal muscle of the cat small intestine is 0·038 m/s (Burnstock & Prosser, 1960) and the corresponding value in the longitudinal muscle of the guinea-pig jejunum 0·032 m/s and, in the presence of tetrodotoxin, 0·021 m/s (Kuriyama *et al.*, 1967). Conduction velocity in smooth muscle is dependent on the diameter of the functional bundle (Burnstock & Prosser, 1960) ; the difference between the conduction velocity found in our experiments, in which the smooth muscle is excited by the transmitter, and that observed in the experiments in which the muscle is excited directly, may be due to differences in the diameter of the functional bundle.

When the release of acetylcholine by the myenteric plexus-longitudinal muscle preparation is determined in the presence of eserine, field stimulation by short trains of pulses causes a greater output of acetylcholine per stimulus than single pulses administered with the same time interval as that between the trains of pulses (A. A. Waterfield, unpublished observations). It is probable that the same relationship holds for the experiments described in this paper when the non-eserinized preparation is stimulated by discrete electrodes. Since in the guinea-pig ileum no nerve fibres enter the longitudinal muscle layer (Paton & Zar, 1968 ; Gabella,

1970), it is possible that the increased amount of acetylcholine released from the myenteric plexus will excite more muscle cells and thus increase the diameter of the functional bundle. Alternatively, such an increase in conduction velocity of the smooth muscle could be simulated by an increase in the distance over which the nervous elements are conducting. The evidence available at present is insufficient to distinguish between these two possibilities although the linearity of the plot of latency of the smooth muscle response against conduction distance favours the first alternative.

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