J. Physiol. (1962), **162**, *pp.* 76–92
With 7 text-figures
Printed in Great Britain

THE ELECTRICAL AND MECHANICAL RESPONSES OF INTESTINAL SMOOTH MUSCLE CELLS TO STIMULATION OF THEIR EXTRINSIC PARASYMPATHETIC NERVES

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(*Received* 17 *November* 1961)

Our understanding of the process of chemical transmission has been greatly aided by information obtained by the use of micro-electrodes inserted into single cells and recording changes in membrane-potential produced by a nerve volley. Information on cholinergic transmission has always been in advance of that for other types of junction chiefly because of the relative ease of recording from skeletal muscle. Other cholinergic junctions, while technically more difficult to examine with intracellular micro-electrodes, have been tackled successfully, so that information on the electrical events at the post-synaptic membrane accompanying neuro-effector transmission is available for skeletal muscle (Del Castillo & Katz, 1956), cardiac muscle (Hutter & Trautwein, 1956), sympathetic ganglia (Eccles, 1955) and secretory gland cells (Lundberg, 1956). In this list there is one conspicuous omission, smooth muscle. There is as yet no information on the events taking place at the membrane of single smooth-muscle cells following the arrival of a nerve volley in the parasympathetic (cholinergic) nerves.

The present paper describes the response of the smooth-muscle cells of the longitudinal coat of the rabbit colon to stimulation of the extrinsic parasympathetic nerves. Some of the results have previously been briefly reported (Gillespie, 1960, 1961).

METHODS

The apparatus and methods used have been described in detail in the previous article (Gillespie, 1962). In brief, rabbits of either sex in the weight range 1·6–3·5 kg were killed by a blow on the back of the neck and bled out. The abdomen was opened in the mid line and the pelvis split. The extrinsic sympathetic (lumbar colonic) and parasympathetic (pelvic) nerves serving the colon were isolated and a 4 cm length of terminal colon, with these nerves attached, was removed. During the dissection the tissues were kept cold by frequent application of chilled Krebs's solution. The isolated piece of colon was opened out flat by a longitudinal cut along its antimesenteric border. Each end of the flat preparation

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was then fixed in a small nylon clamp and the clamped preparation was removed to an isolated organ bath containing a modified Krebs's solution at 36° C. The composition of the saline solution was given previously (Gillespie, 1962): it was oxygenated with 95% O_2 + 5% CO_2.

In the organ bath one clamp was attached to a lever bearing on an electronic isometric recording device (RCA 5734 valve) the output of which was displayed on one beam of an oscilloscope. The other clamp was attached to a fixed arm mounted on a screw by means of which the longitudinal muscle coat of the preparation could be stretched. The micro-electrode was introduced into the longitudinal muscle layer by the floating micro-electrode technique of Woodbury & Brady (1956). The signal from the micro-electrode was led through a cathode follower and displayed on the second beam of the oscilloscope. The nerves were stimulated electrically under a layer of liquid paraffin by 1 msec pulses at voltages supra-maximal for the tissue response. Stimulation of the nerves was signalled by a neon lamp mounted in front of the cathode-ray tube of the oscilloscope.

The micro-electrodes used were of high resistance, optimally between 30 and 70 MΩ. Some records were made with large micro-electrodes (10–15 μ tip diameter).

RESULTS

The effect of single stimuli and slow trains of stimuli to the parasympathetic nerves

Single stimuli or slow trains of stimuli to the parasympathetic motor nerves (the pelvic nerves) were followed by both mechanical and electrical responses. This is shown in Fig. 1. The basic electrical response was a slow prolonged depolarization following the stimulus artifact by an interval of some 400 msec; the response itself lasted about 600 msec. As a purely descriptive term this will be referred to as a 'slow potential ' (s.p.). It is presumably a junctional potential and corresponds to that described as such by Burnstock & Holman (1961) for the smooth muscle of the guinea-pig vas deferens in response to hypogastric-nerve stimulation. If this s.p. was large enough it gave rise to a spike potential, the s.p. then appearing as a pre-potential (Fig. 1). The membrane potential at which the s.p. initiated a spike showed considerable variation; sometimes spikes appeared after the peak of the slow depolarization. The origin of these variations is not known. Some may be due to summation at the smooth-muscle membrane of the depolarization produced by the nerve impulse with other excitatory influences, perhaps derived from neighbouring muscle cells. If there is a large after-hypolarization following the spike, this may temporarily abolish the s.p. and carry the potential back to the base line. The s.p. in these instances usually re-establishes itself (Fig. 1*C*). The delay of 400 msec between the stimulus artifact and the beginning of the s.p. appears to be too long to be accounted for in terms of conduction along the pelvic nerves plus transmission at one synapse in Auerbach's plexus.

One purpose of the present investigation was to see if the responses of the smooth-muscle cells fell into two groups, one of which might correspond

with directly innervated cells, and the other with cells excited either from the directly innervated cells or by overflow of transmitter from adjacent nerve endings at high frequencies of stimulation. No evidence for such a division was found. In unfatigued preparations in which single stimuli were effective all cells penetrated responded to each stimulus. Moreover, the delay of 400 msec was remarkably uniform, i.e. there was no indication of conduction over several units.

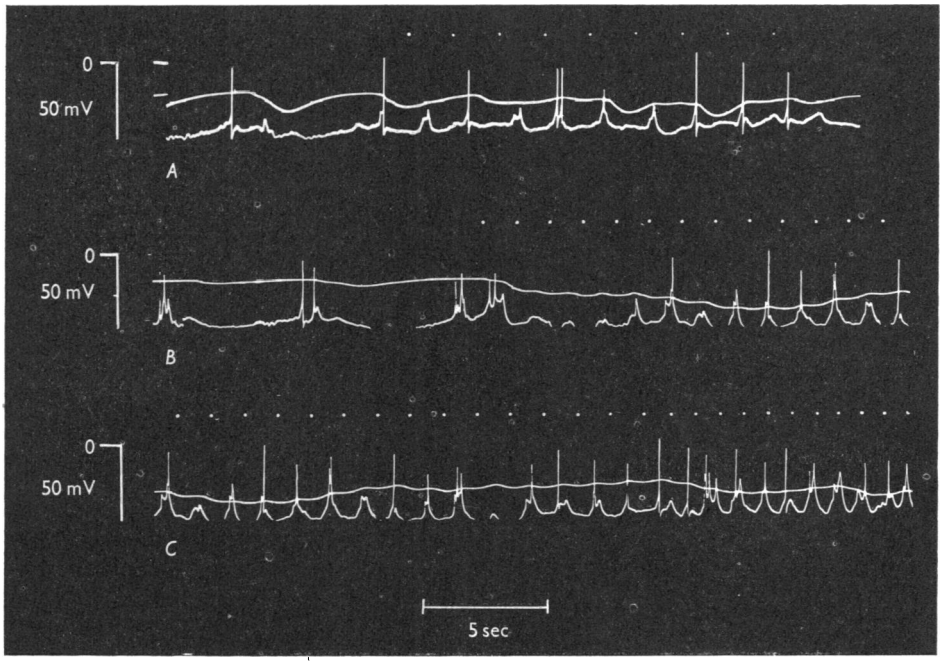

Fig. 1. Records of total tension (upper line) together with the membrane potential of single smooth-muscle cells (lower line) showing the driving effect of slow stimulation of the motor nerves on three different cells. Stimulation of the parasympathetic (motor) nerve indicated at the top of each record by white dots. Each stimulus produces an electrical response and an increment in tension. The electrical response may be either a slow depolarization or, more usually, a spike potential. For further explanation see text.

Changes in excitability can obscure this underlying simple pattern of response. Three circumstances in particular were noted. First, the phase of the spontaneous slow waves determined the size of the s.p. and the likelihood of spike initiation. A nerve volley arriving near mid cycle, i.e. at the phase of maximal repolarization, was less likely to initiate a spike potential than was one which just preceded the spontaneous spikes. This intermittent suppression of spike formation corresponding to the cyclical variations in membrane stability can be seen in Fig. 1 *B*, *C*. Secondly, there

was an increase in excitability during a slow train of stimuli, so that the s.p.s progressively increased in size even though the interval between spikes was apparently sufficient to allow the previous electrical response to disappear. Finally, there was a progressive increase in excitability with time for the first hour or so after setting up the preparation in the bath. The changes in excitability with time (*a*) during a train of stimuli, and

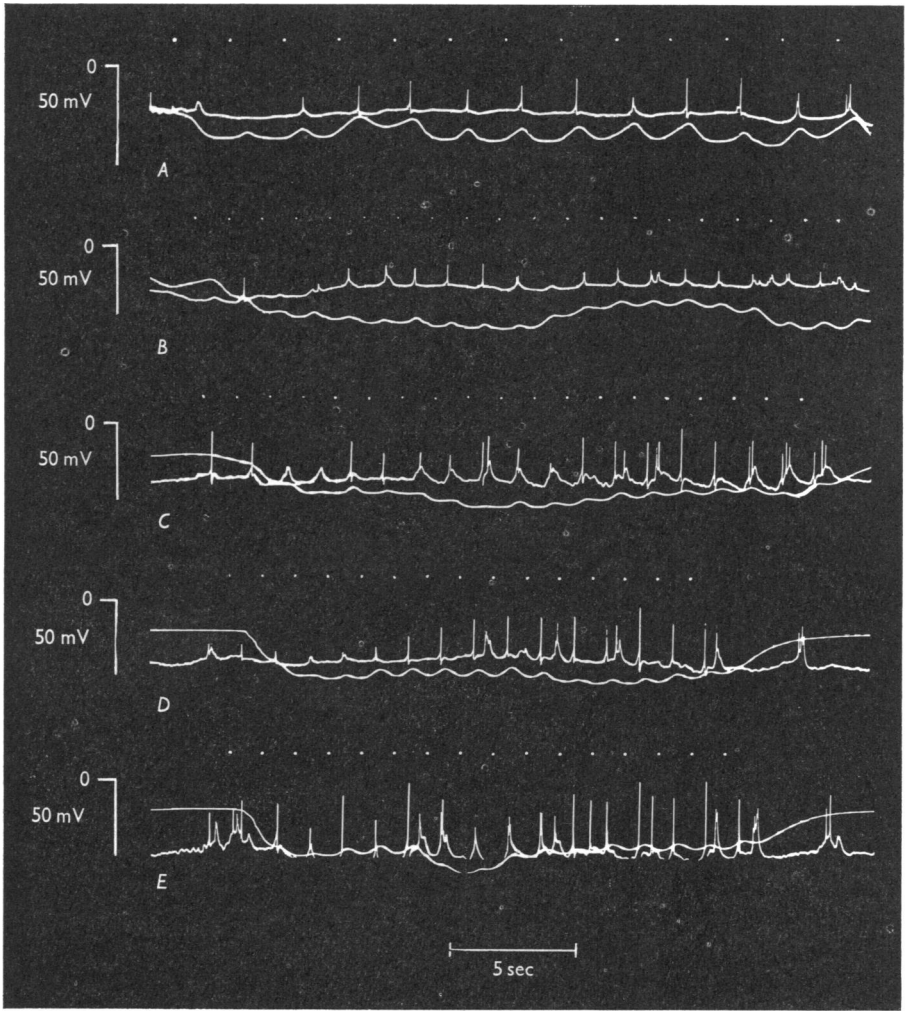

Fig. 2. Records of membrane potential from five cells in the same preparation together with the tension of the preparation, showing the response to slow trains of stimuli to the motor nerve. Nerve stimulation is indicated by white dots. Each record shows the facilitation of the electrical response during a slow train of stimuli. Comparison of successive records shows the increase in excitability with time after the preparation was suspended in the organ bath. For further explanation see text.

(*b*) during the first hour or so of the experiment are shown in Fig. 2. This increase in excitability sometimes produced an irregular discharge of spike potentials apparently unrelated to nerve stimulation. The origin of this irregular discharge, illustrated in Fig. 2*D*, *E*, lies in the appearance of a second spike some considerable time after the first spike response. In 2*D* the extra spikes are easily identified and the responses are clearly related to stimulation. In 2*E* this same phenomenon gives rise to a confused picture because the doubling of the discharge is intermittent and the relation to nerve stimulation therefore obscured. Such a picture if presented alone might easily prevent recognition of the relationship between nerve stimulation and the electrical events. It was noticeable that these variations in excitability affected only the electrical responses of the individual cells, there was no correlation with the mechanical response. The increments of tension following each nerve volley were fairly equal whatever the electrical response and even when the penetrated cell showed little or no electrical response (Fig. 2*A*, *B*). While the tension increments per stimulus were fairly equal the total tension sometimes showed large, slow fluctuations (Fig. 2*B*).

The effect of repetitive stimulation at high frequency

The ability of the smooth-muscle cells to follow increasing frequencies of stimulation was limited. This is shown in Fig. 3, in which the pelvic nerves were stimulated with a train of pulses commencing at about 1/sec then

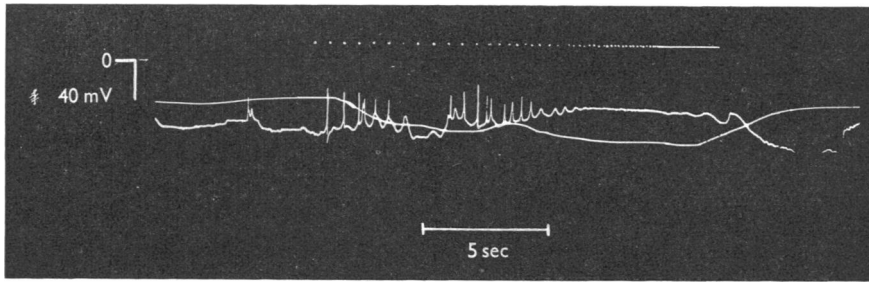

Fig. 3. Membrane potential (lower line) from a single cell together with the tension of the preparation (upper line) during stimulation of the motor parasympathetic nerve at a frequency increasing from 1/sec to 12/sec, shown by the white dots. As the frequency rises above 2/sec the membrane depolarizes, spike potentials diminish and eventually disappear, but tension remains maximal.

steadily increasing to 12/sec. Initially the smooth-muscle cell followed the stimuli in a one-for-one manner, but at a frequency of just less than 2/sec this relationship was lost and the spike potential became irregular. At this same point the base line began to rise, i.e. the cell depolarized and as this continued the spike potentials became smaller, changed to a damped

oscillation and eventually disappeared, leaving a steady, apparently stable state of depolarization. At this time tension of the muscle remained at its maximum. This finding, that the muscle could remain in strong contraction in the absence of any spike potentials but with a depolarized cell membrane, was confirmed repeatedly.

This interpretation may be objected to, on the grounds that, in penetrating these small cells with a micro-electrode, the individual cells may have been so damaged as to be incapable of following high frequencies of stimulation although they were still capable of responding to single stimuli or of following slow trains. If this were true, then external electrodes, which avoided damage to the cells, might still show spike activity at high frequencies of nerve stimulation. This type of recording was therefore attempted. The technique was first to record with an intracellular micro-electrode, then to withdraw the electrode and, under the dissecting microscope, break the tip with fine watchmaker's forceps. The broken micro-electrode was then re-inserted into the tissues and a record taken. When this approach was contemplated it was thought unlikely that any very elegant records would be obtained. Contrary to these expectations it was found extremely easy to get excellent records, such as those shown in Fig. 4. Even more surprising, apart from the amplitude of the signal, these records had all the appearances of those obtained by intracellular recording. On first resting the electrode on the tissue surface there was either no potential change or else a small positive deflexion. If the electrode was then lowered so as to press on the tissues, a resting negative potential of several millivolts developed fairly abruptly, and superimposed on this there were positive-going monophasic spikes. The form of these spikes was identical with that obtained with an intracellular micro-electrode. Complex spikes were no more frequent, in contrast to what might be expected if recordings were from a relatively large number of units.

The ability of smooth-muscle cells to follow increasing frequencies of stimulation of the pelvic nerve as recorded with these large electrodes is shown in Fig. 4. The first record, obtained with a high-resistance micro-electrode, is a repeat of Fig. 3. Following this the electrode was broken, causing a fall in its resistance from 50 MΩ to less than 1 MΩ. The broken micro-electrode was then re-inserted into the tissues. Figure 4*B* shows that with suitable amplification it is possible to demonstrate both s.p.s and the spikes they give rise to, with such an extracellular electrode. Figure 4*C* shows the effect of increasing the frequency of stimulation from 1/sec to 12/sec. The record is almost identical with that obtained with the intracellular electrode, the cell initially following the stimulation with spikes; then at about 2/sec the spikes become irregular, the base line rises

as the cell depolarizes; the spike potentials diminish and disappear leaving a steady, maintained depolarized state. At this time tension remains maximal. The last record in Fig. 4 is interesting in that it shows the fatiguing effect of the previous high-frequency stimulation. Repetition of the slow train of stimuli, each of which in 4*B* produced spike potentials, now produced only s.p.s with a gradual rise in base line.

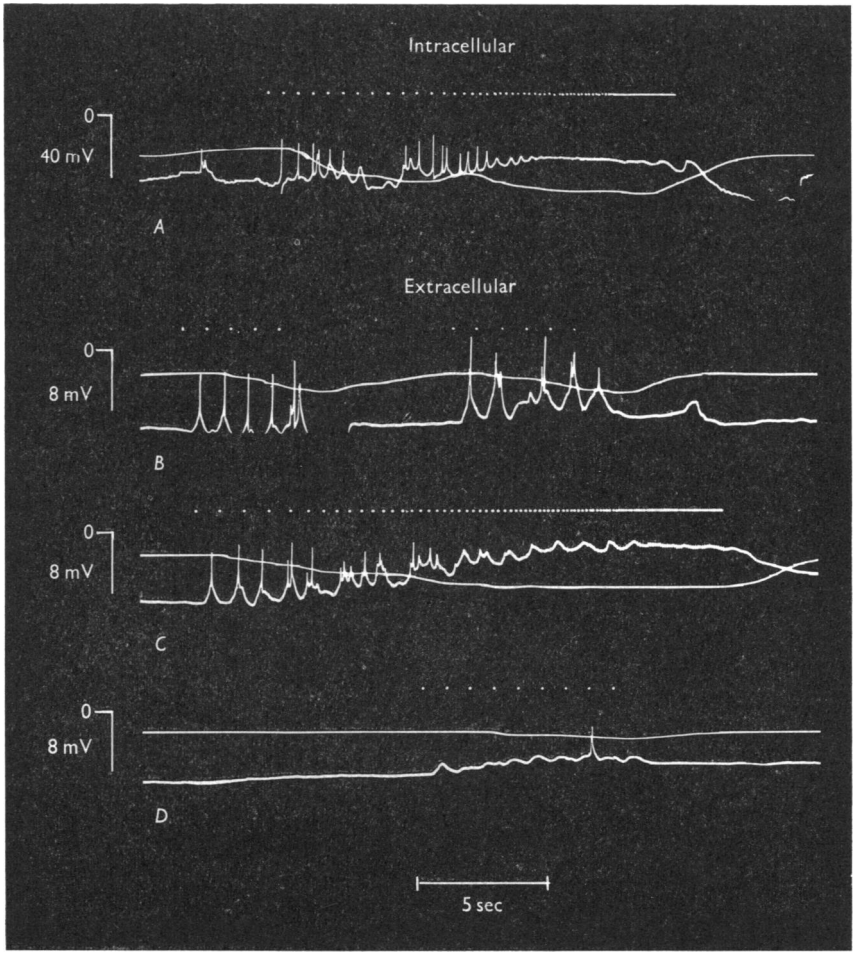

Fig. 4. Records of tension(upper line) and membrane potential (lower line) obtained with either high-resistance (intracellular) or broken (extracellular) micro-electrodes. *A* is a repeat of Fig. 3 with an intracellular micro-electrode. *B*, *C* and *D* were all taken with a low-resistance broken electrode. *B* shows the 'negative resting potential' and positive-going spikes in response to slow stimulation of the motor nerve (the base line was raised between the two periods of stimulation). *C* shows the response to a train of stimuli increasing from 1/sec to 12/sec and *D* shows the fatiguing effect of this fast stimulation. For further explanation see text.

The facility with which recordings could be obtained with these broken micro-electrodes was surprising, and their similarity to those obtained with high-resistance electrodes disturbing. Further experiments were therefore performed to determine the mechanism involved. The possibility that the micro-electrodes, in spite of being broken, were in fact intracellular was

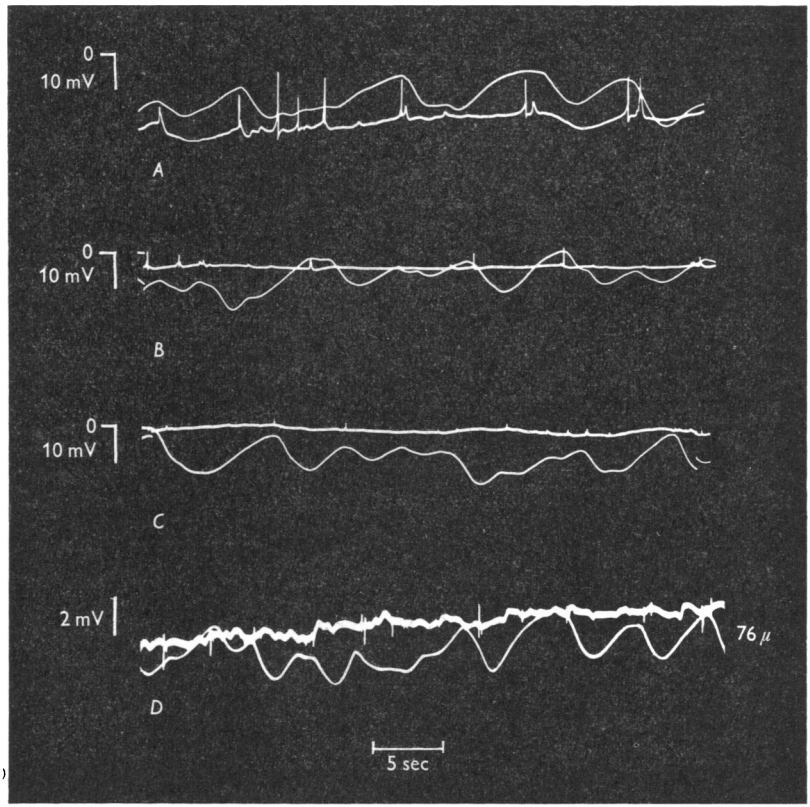

Fig. 5. Record of muscle tension together with membrane potential recorded with a broken (extracellular) micro-electrode. The electrode had been broken once before record *A* was taken. After each recording it was broken again with two results: first a progressive reduction in signal size; secondly, in *D* there is also a change in the character of the record—the resting potential disappears, the signals become biphasic and mainly negative-going. The electrode size measured after *D* was 76 μ.

intrinsically unlikely with such small cells. This was re-inforced by the observation that recording with these broken micro-electrodes, unlike their high-resistance parents, required no delicacy in manipulation. The weight of the micro-electrode was simply allowed to press on the tissue, using the coarse adjustment of the micro-manipulator. Moreover, recording could be continued for long periods, 30–40 min, with

little diminution in the recorded potentials, a sharp contrast with intracellular recording. Optical measurement of the electrode tip diameters finally excluded any possibility of intracellular penetration. The external diameters varied from about 10 to 15 μ (Fig. 6), in all instances greater than the maximal diameter of the smooth-muscle cells. The size of the potentials picked up could be roughly correlated with the tip size (Figs. 5, 6). In one experiment the process of breaking the tip was repeated several times, recording after each break. The result is shown in Fig. 5. There was a progressive diminution in the amplitude of the signals from about 20 mV to about 1 mV. Preceding the final record a fairly large part of the tip was broken off. This not only further reduced the amplitude of the signal but also altered its character. There was now no resting potential and the small spike-potentials were biphasic and mainly negative-going; the type of recording to be expected from an extracellular electrode. The tip diameter was measured at this point and was found to be 76 μ.

The tip diameter of these broken electrodes was considerable. It was possible that sufficient KCl diffused from them to create a local area of depolarization and that it was the resultant demarcation potential which was recorded. That this is not the explanation was shown by filling electrodes with the same Krebs's solution as was used in the bath. Such saline-filled electrodes gave as good records as those filled with 3M-KCl (Fig. 6).

Whatever the origin of the potentials recorded with these broken electrodes, it is clear that the electrodes were not lying inside the cells. From the stability and duration of the recordings it is unlikely that the cells had been much damaged. Therefore the records showing absence of spike potentials with maintained maximal tension at high frequencies of nerve stimulation probably do represent the true state of the population of smooth-muscle cells.

DISCUSSION

The response to parasympathetic-nerve stimulation has brought to light many interesting and some puzzling features. It is clear in these recordings that a single stimulus can cause both a mechanical and an electrical response and that slow trains of stimuli can 'drive' the preparation. The increment of tension produced in the tissue by each nerve volley is much more constant than the electrical response of any particular cell penetrated by the micro-electrode (see Figs. 1, 2). Presumably each nerve volley will cause an increment in membrane depolarization which, in those cells already near the critical firing level, will be sufficient to cause them to fire and to add their tension to that of those cells which would have been expected to fire in any case. If the resulting increments in tension are equal, then presumably the number of extra muscle cells recruited by each

nerve volley must be equal. The large slow fluctuations in tension which occur independently of the increments of tension attributable to nerve stimulation may represent variations in background spontaneous activity.

During a train of stimuli there is a noticeable facilitation of the electrical response. Similar facilitation was reported by Burnstock & Holman (1961) in the vas deferens with trains of stimuli as slow as 1/sec. Either there is

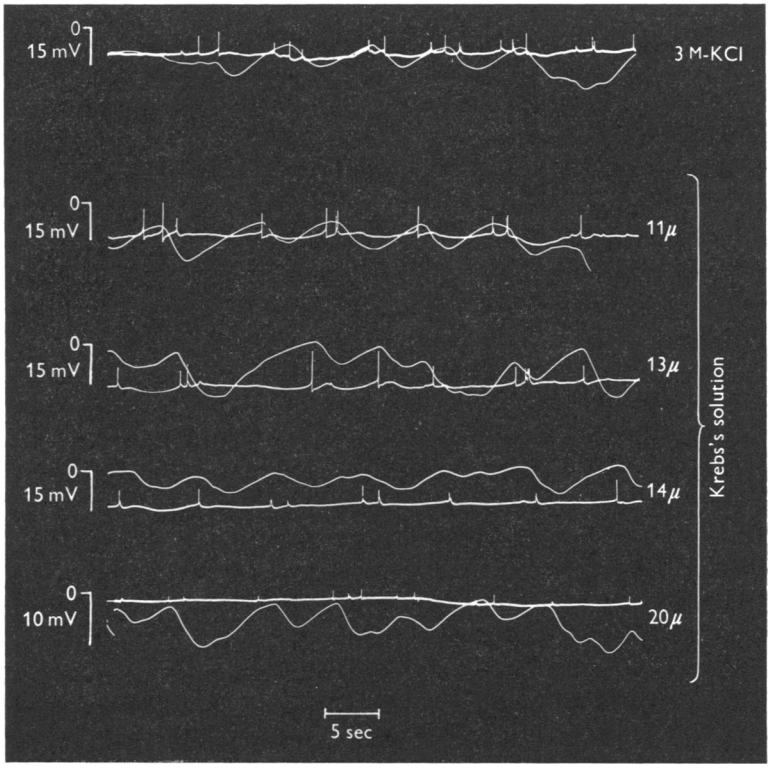

Fig. 6. Records of muscle tension together with membrane potential recorded with broken micro-electrodes. The first record was obtained with a micro-electrode filled with 3 M-KCl. The remaining records were obtained with micro-electrodes filled with Krebs's solution. KCl is clearly not necessary to obtain this type of record. The electrode diameters are shown on the right; there is a rough inverse correlation between signal size and tip diameter.

some residual effect of the liberated transmitter on the membrane which lasts as long as 1 sec, or else some facilitating phenomenon, perhaps related to post-tetanic enhancement, occurs in the nerve.

The timing of the electrical events presents problems which only further experiments can resolve. The interval of 400 msec between the stimulus artifact and the beginning of the s.p. seems much too long to be explained

by conduction in the extrinsic pelvic nerves and transmission at the single synapse postulated in Auerbach's plexus. Conduction velocities in the extrinsic nerves are unlikely to be less than 0·5 m/sec; allowing a 6 cm length of pathway one could therefore account for perhaps 120 msec. Transmission at the synapse might, at the most, account for a further 10–20 msec (Eccles, 1943) giving a total of 140 msec, and leaving some 250 msec unexplained. It is unlikely that much time is occupied in events at the nerve endings and at the smooth-muscle membrane itself, since Burnstock & Holman (1961) have shown that the smooth muscle of the vas deferens responds to hypogastric-nerve stimulation after an interval varying from 20–70 msec, of which only about 10 msec is occupied in junctional transmission. The possibilities would seem to be either that the lengths of the nerve pathways inside the wall of the gut are much longer than had been imagined, that conduction in these intramural fibres is much slower than in any fibres at present known, or finally, that there are some other regions of delay in addition to the single synapse postulated in Auerbach's plexus.

The smooth muscle of the vas deferens and that of the colon make an interesting contrast, in that the motor nerve is adrenergic in one (the vas) and cholinergic in the other (colon). The surprising thing is that the electrical response to nerve stimulation is so similar in the two cases. For example, the duration of the s.p. in the colon corresponds very closely with that of the junctional potential reported by Burnstock & Holman (1961). Burnstock & Holman suggested that the long duration was probably due to persistence of the transmitter at the membrane. This would be an attractive hypothesis in the colon also and would explain the re-establishment of the s.p. even after hyperpolarization following a spike had carried the membrane potential down to the previous base line, e.g. some spikes in Figs. 1 and 2. It is surely more than coincidence, however, that both transmitters should persist for a similar length of time at the membrane.

One difference in the responses of these two muscles to nerve stimulation, the need in the vas deferens for summation of several junctional potentials before a spike is initiated, is probably related more to differences in the membrane stability of the smooth-muscle cells than to any difference in the action of the transmitters. In the colon the membrane potential undergoes spontaneous fluctuations and the muscle is rhythmically active. In the vas deferens the membrane potential is stable (Burnstock & Holman, 1961) and the muscle shows no spontaneous activity (personal observation).

In the discussion so far it has been assumed that contraction of a smooth-muscle cell is the consequence of a spike potential. When frequencies greater than 2/sec are used, however, the relationship between spikes and contractions becomes obscure and at 10/sec the smooth-muscle contraction,

which is maximal, is associated with depolarization of the membrane in the absence of any spike potentials, a phenomenon akin to contracture in skeletal muscle on adding excess potassium to the bathing medium. The stimulation frequency at which this phenomenon of maintained depolarization begins to appear (2/sec) corresponds to the beginning of overlap of the s.p.s and may well represent the accumulation of chemical transmitter. A similar disappearance of spike potentials and their replacement by 'damped oscillations', has been noted by Holman (1958) in the taenia coli of the guinea-pig as a response to excess potassium in the external fluid. The present experiments take this observation one step further by demonstrating that this same phenomenon may be produced by physiological means, stimulation of the motor nerve, and at frequencies believed to be within the physiological range for autonomic nerves. Furthermore, the oscillations will progress to a quite steady state of depolarization with no evidence of discrete events. The significance of this observation is difficult to assess. Since the effect can be produced by stimulation of the motor nerve at frequencies believed to be physiological, it may be that contractures of this type do occur during life in intense contraction of smooth muscle. Evidence supporting such an idea is provided by Bozler (1949). Using extracellular electrodes Bozler found that in the ileum of the dog peristalsis is normally accompanied by bursts of spike discharges, but in very strong contractions these may be absent and only a slow negative potential may be seen. A similar disappearance of spike potentials in spite of the continuance of strong contraction has been noted in the nictitating membrane under the action of adrenaline (Eccles & Magladery, 1937). On the other hand, such contractures may never occur physiologically and the proper inference is that the physiological upper limit for the frequency of discharge in autonomic nerves is even less than is commonly believed.

The type of record obtained with large (broken) micro-electrodes was most surprising in that it had none of the characteristics expected of extracellular recording in a volume conductor. Indeed, apart from the size of the signal, the characteristics were those of intracellular recording: there was a resting negative potential, positive-going monophasic spikes and, in appropriate circumstances, slow changes in potential paralleling and in proportion to what would be recorded with an intracellular electrode. Similar records have in fact been obtained from the smooth muscle of the guinea-pig ureter and small intestine, with KCl-filled electrodes with a tip diameter of from 5 to 10 μ (Greven, 1953). The positive-going spikes recorded by Greven diminished over a few minutes and finally reversed to small negative-going spikes. The explanation given was that these relatively large electrodes injured the muscle membrane, causing an injury current to flow. During a spike potential in the neighbouring (undamaged) mem-

brane this injury current was supposed to be reversed, with the result that the injured area under the electrode became less negative. The diminution with time of recorded potentials was attributed to diminution of the injury current, and the eventual reversal to a negative-going spike was held to be due to death of a small area of membrane in the neighbourhood of the electrode. This dead membrane then acted as an extension lead, recording with attenuation the negative-going spikes in neighbouring healthy membrane. While the records reported in the present article are essentially similar to those observed by Greven the explanation he offered does not seem appropriate. In the present experiments the resting negative potential and positive-going spikes did not diminish with time in the periods studied (up to 45 min), nor was reversal to negative-going spikes ever seen. From the long duration of recording that was possible it can be argued that there was little damage to the cell as a whole and there was no evidence that depolarization by K ions was occurring. Electrodes filled with saline were as effective as those filled with 3 M-KCl. In the previous article evidence was given for a depolarizing effect of stretch (Gillespie, 1962). Such a depolarizing effect might explain these unusual recordings with extracellular electrodes. If the muscle membrane is sensitive not only to general stretching, but also to localized mechanical deformation, then an external electrode pressed against the muscle membrane, and producing local deformation, would also produce local depolarization. If one other assumption is made, that a region of high electrical resistance isolates that part of the membrane adjacent to the interior of the micro-pipette from the rest of the external membrane, the recording would theoretically be expected to look something like that obtained with an intracellular micro-electrode. The suggested mechanism is illustrated diagrammatically in Fig. 7 together with the equivalent electrical circuit.

Pressure of the micro-electrode causes a graded increase in permeability of the membrane represented by the variable leak resistance R_L. This short-circuits the membrane potential E and membrane resistance R'_M in this region and causes a local circuit current i to flow. The potential V, recorded between the micro-electrode and the reference electrode, will be the voltage drop over the seal resistance R_S.

$$V = iR_S \tag{1}$$

If we take the special case when R_L goes to zero, then the membrane is completely short-circuited and both the membrane potential and the membrane resistance in this region become zero. Under these conditions the e.m.f. driving i is E and

$$i = \frac{E}{R_M + R_S}. \tag{2}$$

Substituting in eqn. 1,

$$V = E\left(\frac{R_S}{R_M + R_S}\right). \tag{3}$$

When R_L is zero, therefore, the voltage recorded depends on the relationship between R_S and R_M. If R_S is large compared with R_M almost the whole membrane potential will be recorded. If R_L is not zero, then the larger it is, the smaller V, the recorded fraction of the total membrane potential will be.

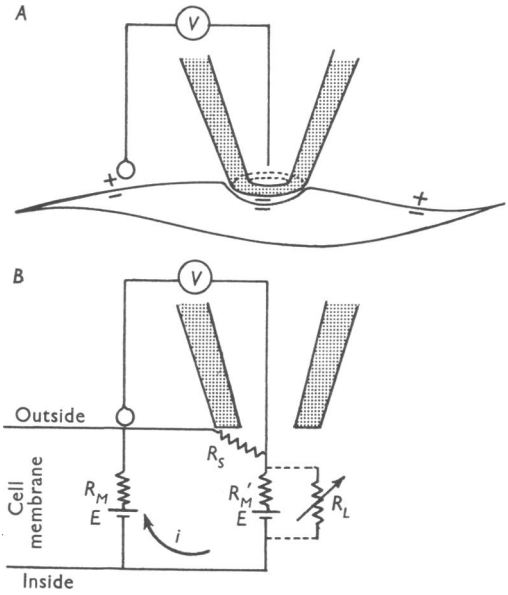

Fig. 7. *A*. Diagrammatic representation of a possible mode of origin of the records obtained with extracellular electrodes. Mechanical deformation by the electrode causes local depolarization. *B*. Equivalent electrical circuit of *A*. For explanation see text.

This theory would explain the negative resting potential seen with these extracellular electrodes as being due to the local depolarization consequent on mechanical deformation. The large size of the signal would be the result of electrical isolation of the muscle membrane under the micro-electrode either by indentation of the surface membrane or by some process of 'sealing' of the electrode to the membrane. Finally, the monophasic positive-going nature of the spikes would be evidence that the spike potentials could not invade the region of membrane under the micro-electrodes. It may be that for this type of recording the floating micro-electrode technique is essential since it allows the micro-electrode to adapt itself to, and move freely with, the muscle surface, thus maintaining this 'seal' between electrode and muscle membrane. The experimental observation that slight pressure is necessary to develop the potentials and often

a slight increase in pressure during recording will increase the signal size is also consistent with this idea.

Whatever the theoretical basis, it is clear that micro-electrodes too large to be intracellular can give records identical, other than in signal size, with those obtained with a supposedly intracellular electrode. This raises the question as to what are the appropriate criteria by which to judge whether an electrode is indeed inside the cell. If, as the experiments suggest, the size of the signal alone distinguishes the records obtained with these large extracellular micro-electrodes, and if the signal increases in size as the tip becomes smaller, at what point does one say that the size of the signal merits considering the micro-electrode as inside the cell? At either extreme it is easy. With high-resistance micro-electrodes, recording potentials of 60 mV or more, it is unlikely that the electrode is anything but intracellular. This belief is reinforced by the delicacy needed in positioning the electrode, by the short duration of recording which is usually possible, and by the steady decline in potential over minutes which is seen with longer penetrations and is thought to indicate deterioration of the cell. With very large (10–15 μ) micro-electrodes of low resistance, recording a few millivolts of potential, requiring the minimum care in placing, stable for long periods and with no evidence of cell deterioration, recording is certainly extracellular. Between these extremes, with electrodes of intermediate size, there are potentials whose origin poses a problem. It is possible that some of the low potentials which occur in many reported investigations of smooth muscle are due to electrodes which have not penetrated the cell membrane. It was to exclude this possibility that, in measuring membrane potentials, an arbitrary minimal electrode resistance of 20 MΩ was used as one necessary criterion for satisfactory penetration (Gillespie, 1962).

While the possibility of obtaining such records with extracellular electrodes is inconvenient when measuring the absolute values of the membrane potential, it may offer a very useful technique when such absolute values are not required. In the study of drug action, for example, where records of longer duration are required than can easily be obtained with intracellular electrodes, this technique might well be a convenient alternative to the sucrose-gap technique of Burnstock & Straub (1958). The present method is extremely simple and no skill is required in placing the electrodes. If electrodes were deliberately pulled with tip diameters of about 5 μ then almost certainly still larger potentials would be recorded. As it is, one can easily obtain 10–15 mV resting and spike potentials and observe changes in both, proportional to those recorded with intracellular micro-electrodes.

SUMMARY

1. The effect of parasympathetic (motor) nerve stimulation on the membrane potential of single smooth-muscle cells and on the tension of the preparation as a whole has been studied *in vitro* in an innervated preparation of rabbit colon.

2. Single stimuli to the parasympathetic (motor) nerve caused both a mechanical and an electrical response. The basic electrical response was a depolarization following the stimulus artifact after an interval of about 400 msec and itself lasting about 600 msec. This has been referred to as a slow potential (s.p.) The size of the s.p. depended on the phase of the slow wave at which it occurred. It was smallest at the time of maximal repolarization and increased as the cell spontaneously depolarized. If large enough the s.p. gave rise to a spike potential. The interval of 400 msec seems too long to be explained by conduction in the extrinsic nerves and transmission at one synapse.

3. The electrical response of single cells to trains of stimuli as slow as 1/sec showed facilitation.

4. The ability of individual smooth-muscle cells to follow increasing frequencies of nerve stimulation with spike potentials was limited. At about 2/sec the cell began to depolarize and the one-for-one relationship between stimuli and spikes was lost. With increasing frequency of stimulation the depolarization increased, the spikes diminished in height and became broader, gradually changing to a slow oscillation which at a frequency of about 10/sec disappeared leaving a steady depolarized membrane. In spite of the absence of spikes, tension remained high.

5. Some observations were made with relatively large (10–15 μ) glass micro-electrodes. The nature of the record obtained with such electrodes is discussed.

This work was done in the Rockefeller Institute, New York, and I should like to acknowledge the help and assistance received there; in particular to thank Dr C. M. Connelly for helpful advice and discussion and Dr F. Brink for the kind hospitality of his laboratory.

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