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TRANSMISSION FROM PERIVASCULAR INHIBITORY NERVES TO THE SMOOTH MUSCLE OF THE GUINEA-PIG TAENIA COLI

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SUMMARY

1. Membrane potential changes of the smooth muscle cells of the taenia coli were recorded during stimulation of the perivascular inhibitory nerves.

2. Some spontaneous action potentials were preceded by a slow pacemaker-like potential. Others began at or near the maximum level of the membrane potential and were not preceded by pacemaker-like potentials.

3. There were no changes in the membrane potential of smooth muscle cells when the inhibitory nerves were stimulated with a single pulse. Stimulation at frequencies greater than 5 pulses/sec caused a hyperpolarization of the smooth muscle membrane. This resulted in a decrease in spike frequency and relaxation.

4. When the frequency of stimulation of the inhibitory nerves was increased there was an increase in the amplitude and rate of rise of the hyperpolarization and a decrease of the latency. The latency varied from 150 to 300 msec, and the largest hyperpolarization recorded was 16 mV.

5. The effect of the hyperpolarization due to nerve stimulation in cells showing pacemaker-like activity was to increase the level of the membrane potential at which the action potentials began and to increase the membrane potential to which the action potentials repolarized. Action potentials which occurred during hyperpolarizations of the membrane had greater rates of rise and fall and larger amplitudes than did the action potentials which occurred before hyperpolarization.

6. The effect of the hyperpolarization due to nerve stimulation in cells which did not show pacemaker-like activity depended on the amplitude of the hyperpolarization. Small hyperpolarizations exposed small depolarizations of the membrane which occurred when an action potential would normally have been expected. Large hyperpolarizations blocked the action potentials entirely.

7. Action potentials did not begin firing again at the normal rate immediately after stimulation ceased. The time taken for the rate of firing of action potentials to return to normal increased with increasing frequency of stimulation.

8. The hyperpolarization in response to perivascular inhibitory nerve stimulation was blocked by guanethidine and bretylium.

INTRODUCTION

Electrophysiological studies of transmission from autonomic nerve to smooth muscle have been mainly concerned with the transmission of excitation. Excitatory junction potentials have been recorded and analysed in the following preparations: guinea-pig vas deferens-hypogastric nerve (Burnstock & Holman, 1961); rabbit bladder strip-nerve preparation (Ursillo, 1961); rabbit distal colon-pelvic nerve (Gillespie, 1962*b*); dog retractor penis-sympathetic nerve (Orlov, 1962); guinea-pig mesenteric artery-sympathetic nerve (Speden, 1964). The only study of the transmission of inhibition from autonomic nerve to smooth muscle is that of Gillespie (1962*a*) who worked with the sympathetic supply to the distal colon of the guinea-pig. Whereas depolarizations were recorded at excitatory junctions in response to single pulses, Gillespie (1962*a*) did not record hyperpolarizations until the nerves were stimulated at frequencies greater than 10 pulses/sec.

This paper describes the electrophysiological characteristics of transmission from perivascular inhibitory nerves to the smooth muscle of the taenia coli of the guinea-pig. In general our results are similar to those described by Gillespie for the distal colon. Hyperpolarization of the cell membrane was not detected until the frequency of stimulation was greater than 5/sec. In addition to studying the effects of different frequencies of stimulation we have made some observations on the effect of hyperpolarization of the cell membrane on the configuration of the action potentials in the taenia coli.

METHODS

Guinea-pigs of either sex, weighing 200–500 g, were used. The animals were stunned and bled to death. The taenia coli with its perivascular nerves was dissected in a similar manner to that described by Burnstock, Campbell & Rand (1965). The taenia was mounted on a Perspex block 3 cm long and 1 cm wide, with the serosal surface uppermost, and the block was secured in a 10 ml. rectangular bath containing modified Krebs's solution (Bülbring, 1953). Both ends of the taenia coli were tied with cotton, one piece of which was passed through a pulley to a Grass tension transducer and the other piece was secured to the Perspex block. The perivascular nerves were passed through two loops of platinum wire 1 mm in diameter which were separated by 2 mm and partly embedded in Araldite. The nerves passed through the wire loops at a distance of about 2 cm from the taenia coli.

The solution in the bath was continually replenished from a reservoir where it was bubbled

with 95% O_2 and 5% CO_2, and was maintained at a temperature between 34 and 37° C. This solution was maintained at a level 1 mm above the surface of the preparation.

A Grass model 4 stimulator was used to deliver monophasic square wave pulses of 200 μsec duration at different frequencies to the platinum stimulating electrodes. Glass microelectrodes were filled with 2 M-KCl and usually had a resistance of between 40 and 70 MΩ. These electrodes were mounted flexibly in the manner described by Woodbury & Brady (1956). A transistorized pre-amplifier of the type described by Pugsley (1963) in conjunction with a Textronix 502 A oscilloscope were used for recording. Observations were recorded on moving film with a Grass camera.

The solution in the bath always contained atropine sulphate (10^{-7} g/ml.) so the effects of stimulating cholinergic excitatory nerves were eliminated.

RESULTS

Normal spontaneous activity

Preparations were set up isometrically under several grams of tension, in the presence of atropine (10^{-7} g/ml.). The records described in this and the subsequent paper (Bennett, Burnstock & Holman, 1965) were from cells which fired action potentials at regular intervals. The frequency of firing ranged from one spike every 0·9 sec to one every 6 sec in different preparations. This variation was probably due to different preparations being stretched by different amounts (Bülbring & Kuriyama, 1963). The action potentials recorded from our preparations were similar to those described by Holman (1958) and many had inflexions on their falling phase and sometimes on their rising phase.

In this paper we will describe the effect of perivascular nerve stimulation on two contrasting types of activity. The first of these, which is illustrated in Fig. 1*a*, consisted of action potentials which were preceded by slow depolarizations. The maximum level of the membrane potential was reached by the repolarization phase of the action potential and this was followed by a slow depolarization which led directly into the next action potential. This activity shows many similarities with that of cardiac pacemakers and will be referred to as 'pacemaker-like' activity.

The second type of activity to be described in this paper is illustrated in Fig. 1*b*. In these cells the repolarization phase of the action potentials was slow and often continued until the next action potential was initiated. The maximum level of the membrane potential was therefore reached immediately before an action potential. The absence of a slow depolarization before action potentials and the initiations of action potentials at a high membrane potential suggest that these cells may be 'driven' by activity generated by pacemaker cells in their vicinity. This activity will be referred to as 'driven' activity.

Other types of activity intermediate between the two types described above were also observed. For instance, if the interval between successive action potentials was long (more than 2 sec) the slow changes in mem-

brane potential between successive action potentials was variable. The slow repolarization phase of the action potentials was sometimes followed by a period of constant membrane potential. In some cases the action potentials arose from depolarizations which were not related to the falling phase of the preceding action potential.

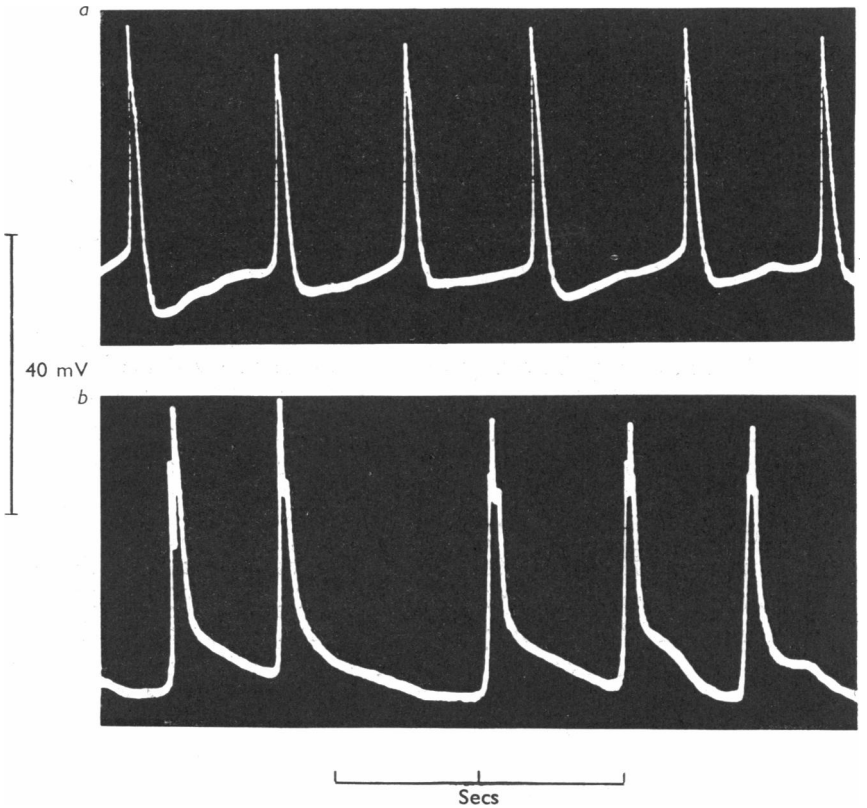

Fig. 1. Two contrasting types of activity recorded in the smooth muscle cells of the taenia coli. (*a*) Action potentials proceded by slow depolarizations ('pacemaker-like' activity). Cell 55. (*b*) Action potentials with slow repolarization phases ('driven' activity). Cell 64. Action potentials retouched.

Perivascular inhibitory nerve stimulation

There was no change in the membrane potential of smooth muscle cells or the rate of firing of action potentials when the perivascular inhibitory nerves were stimulated with a single pulse. When the perivascular nerves were stimulated repetitively at frequencies above about 5 pulses/sec there was a hyperpolarization of the membrane and a decrease in the rate of firing of action potentials. The effect of this hyperpolarization depended on the type of activity occurring in the cell.

Effect of inhibitory nerve stimulation on 'pacemaker-like' activity. In these cells stimulation of the perivascular nerves caused a hyperpolarization of the cell membrane whose characteristics depended on the frequency of stimulation. There was a decrease in the time between the beginning of stimulation and the beginning of hyperpolarization, i.e. a decrease in latency, with an increase of the frequency of stimulation. This is illustrated in Fig. 2, where the latency is shown to decrease from 250 msec at 50 pulses/sec to about 150 msec at 80 pulses/sec. The rate of

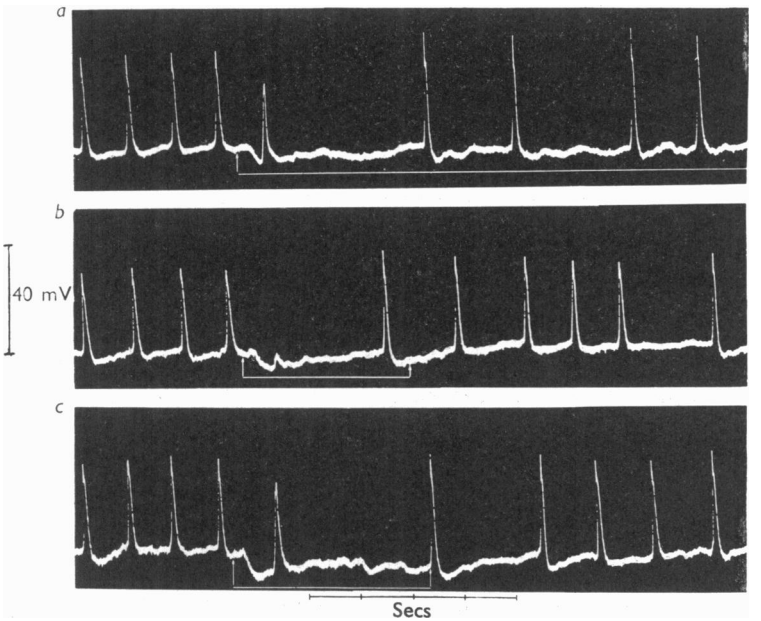

Fig. 2. The effect of different frequencies of stimulation of the perivascular inhibitory nerves on the membrane potential of a cell showing pacemaker-like activity. Frequencies of stimulation 50, 60 and 80 pulses/sec in *a*, *b* and *c*, respectively. Nerves stimulated for the period indicated by the arrows. Pulse duration 200 μsec. Cell 55.

hyperpolarization of the membrane increased with increasing frequency of stimulation. This is also shown in Fig. 2, where the rate of hyperpolarization at 50 pulses/sec was 25 mV/sec but at 80 pulses/sec this increased to 60 mV/sec. The hyperpolarization reached a maximum amplitude a few hundred milliseconds after the beginning of stimulation and was maintained at about this level during stimulation. The maximum amplitude of hyperpolarization increased with increasing frequency of stimulation, as shown in the records of Fig. 2. Figure 3 shows that the hyperpolarization of one cell increased from zero at 1 pulse/sec to 9 mV at 80 pulses/sec. There is then an increase of the amplitude and rate of rise

of hyperpolarization and a decrease of the latency with an increase of the frequency of stimulation.

The effect of the hyperpolarization due to perivascular nerve stimulation on cells showing pacemaker-like activity was to increase the level of the membrane potential at which the action potential commenced and to increase the membrane potential to which the action potential repolarized.

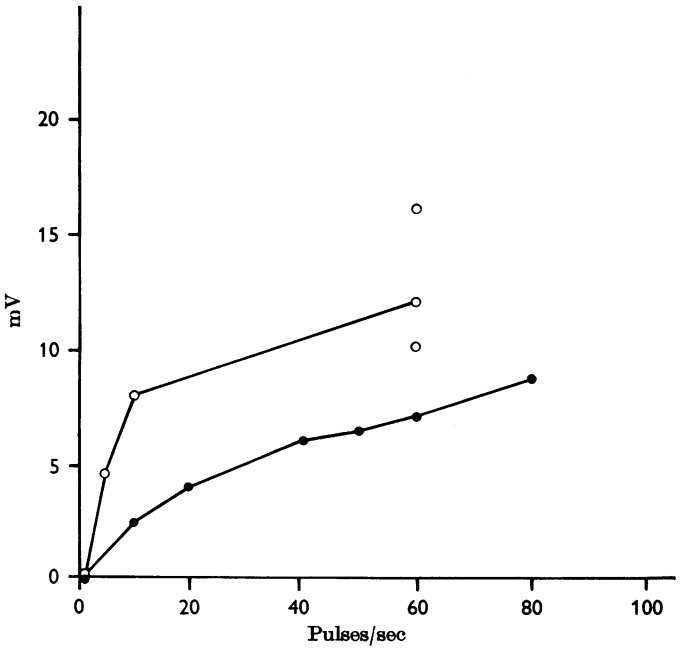

Fig. 3. Changes in amplitude of the hyperpolarization caused by perivascular inhibitory nerve stimulation at different frequencies. Abscissa, frequency of stimulation. Ordinate, amplitude of the maximum hyperpolarization. Closed circles, hyperpolarizations of cell 55 which showed pacemaker-like activity. Open circles, hyperpolarizations of cells 63, 64, 66 and 68 which showed driven activity and which were from the same preparation.

This is illustrated in Fig. 2*b*; in this case the action potential which occurred during stimulation commenced at a membrane potential which was 2 mV greater than the level at which action potential commenced before stimulation. This action potential repolarized to a membrane potential 5 mV greater than that reached by the action potentials before stimulation. There was probably a decrease in the rate of depolarization between action potentials during hyperpolarization due to nerve stimulation. However, the fluctuations of the membrane potential made this depression of the depolarization rate uncertain (Fig. 2*c*).

During the first second of hyperpolarization there often occurred a small

or large depolarization of the membrane, whose duration was comparable with the foot of an action potential. An example of a small depolarization is shown in Fig. 2*b*, while a larger depolarization is shown in both Fig. 2*a* and *c*. These potential changes occurred when the hyperpolarization had reached its maximum value and they were not usually preceded by a pacemaker-like depolarization.

The action potentials which occurred during hyperpolarization of the membrane generally had a greater rate of rise, greater rate of fall and larger amplitude than did the action potentials before stimulation. These changes, which are illustrated in Fig. 2, may be due to the decrease of sodium inactivation which probably occurs when the membrane is hyperpolarized, allowing a greater sodium permeability during the rising phase of the action potential (Hodgkin & Huxley, 1952).

Effect of inhibitory nerve stimulation on 'driven' activity. The rate of rise and the latency of the hyperpolarization of the membrane of these cells could not be measured because the negative after-potential continued directly into the beginning of hyperpolarization. It was therefore not possible to tell when the repolarization ended and the hyperpolarization began. However, the amplitude of the hyperpolarization was observed to increase with increasing frequencies of stimulation, as shown in Fig. 4. Figure 3 shows how the hyperpolarization in one preparation increased from zero at 1 pulse/sec to between 10 and 16 mV at 60 pulses/sec.

The effect of the hyperpolarization due to perivascular nerve stimulation on cells which did not show pacemaker-like activity depended on the magnitude of the hyperpolarization. Small hyperpolarizations of the membrane did not entirely block action potential activity and small depolarizations of the membrane were sometimes exposed when an action potential would normally have been expected. This is illustrated in Fig. 4*a*; small depolarizations of the membrane which had similar time courses to that of the foot of the action potentials occurred during stimulation at 4 pulses/sec. Large hyperpolarizations of the membrane due to stimulation at high frequencies blocked action potential activity entirely. This is shown in the record Fig. 4*c* in which there was no spontaneous activity during stimulation at 60 pulses/sec or for several seconds after the cessation of stimulation. The depression of spontaneous activity of this type was clearly dependent on the amplitude of the hyperpolarization and, hence, on the frequency of stimulation of the perivascular nerves.

At the end of repetitive stimulation it took some time for the action potentials to resume the same firing rate they had before stimulation, and this time increased with the frequency of stimulation. This is shown in Figs. 2*b* and 4*c*. Figure 5 shows the times at which action potentials occurred before and after repetitive stimulation at different frequencies.

In one case the nerves were stimulated for 4 sec at 60 pulses/sec and the normal firing rate did not resume until 4 sec after stimulation ceased. In another case, after stimulation of the nerves for 4 sec at 20 pulses/sec, the normal firing rate resumed immediately after stimulation. Figure 5 also shows that when the nerves were stimulated for longer times (10 sec) the interval between the end of stimulation and the resumption of normal firing showed the same frequency dependence.

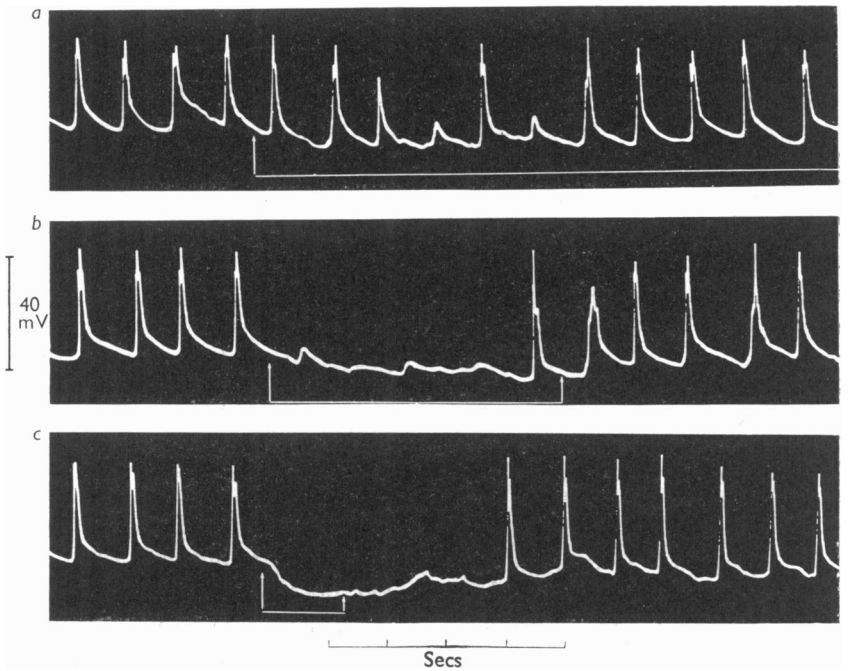

Fig. 4. The effect of different frequencies of stimulation of the perivascular inhibitory nerves on the membrane potential of cells which showed driven activity. Frequencies of stimulation 4, 10 and 60 pulses/sec in *a*, *b* and *c* respectively. Nerves stimulated for the period indicated by the arrows. Pulse duration 200 μsec. *a* and *b* cell 64, *c* cell 67. Action potentials retouched.

In some cases, if the perivascular inhibitory nerves were stimulated just before an action potential began, this action potential had a faster time course than usual. Figure 6*a* illustrates this effect, the perivascular nerves in this case being stimulated at 50 pulses/sec, 280 msec before the beginning of the action potential. Figure 6*b* shows that stimulation of the nerves immediately after an action potential occurred in this cell caused a hyperpolarization of the membrane with a latency of about 280 msec. It is therefore likely that the 'synaptic' currents which normally cause hyperpolarization were flowing during the fast action potential and

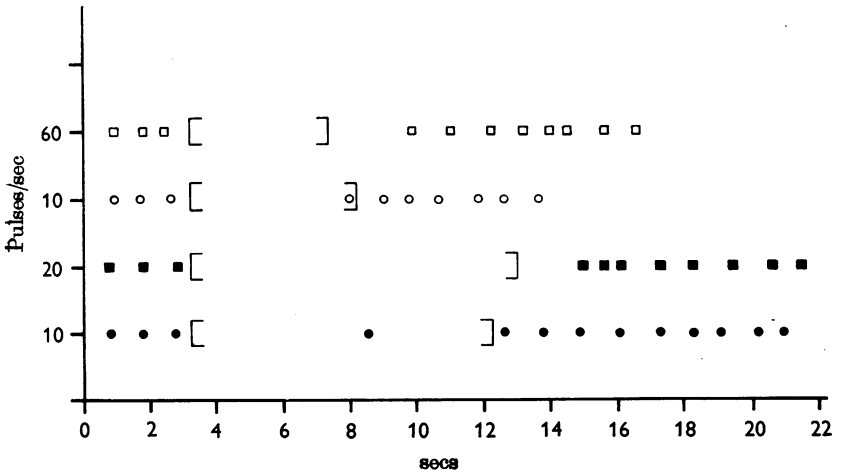

Fig. 5. Times at which action potentials occurred before and after repetitive stimulation at different frequencies. Abscissa, time. Ordinate, frequency of stimulation of the perivascular inhibitory nerves. Square brackets give the period of stimulation at each frequency. Open squares, open circles, closed squares and closed circles give the intervals between action potentials in cells 68, 62, 64 and 52, respectively.

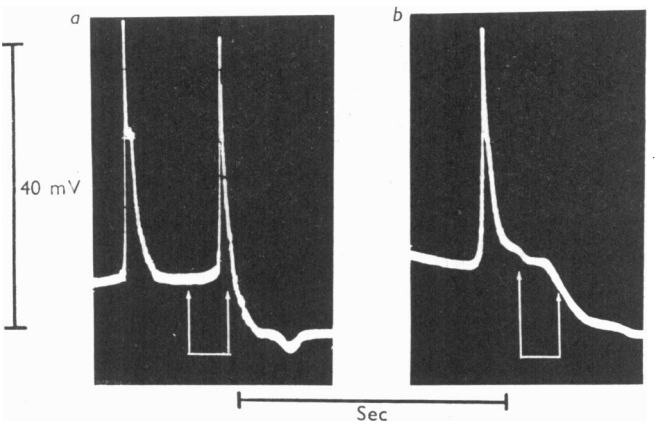

Fig. 6. Fast action potentials occurring at the beginning of stimulation of the perivascular inhibitory nerves. (*a*) Increase in the rate of repolarization of an action potential which occurred 280 msec after the beginning of stimulation of the perivascular nerves at 50 pulses/sec. (*b*) Stimulation of the perivascular nerves at 50 pulses/sec just after an action potential. Arrows indicate a period of 280 msec after the beginning of stimulation. Pulse duration 200 μsec. Cell 50. Records retouched.

that these currents had the effect of increasing the speed of repolarization of the action potential. Kandel, Spencer & Brinley (1961) give a similar explanation for the increase in speed of the antidromic action potential recorded in hippocampal neurones after fimbrial stimulation. In this case the arrival of the action potential at the soma is coincident with the beginning of hyperpolarization due to collateral inhibition and hence the synaptic currents assist the repolarization of the action potential.

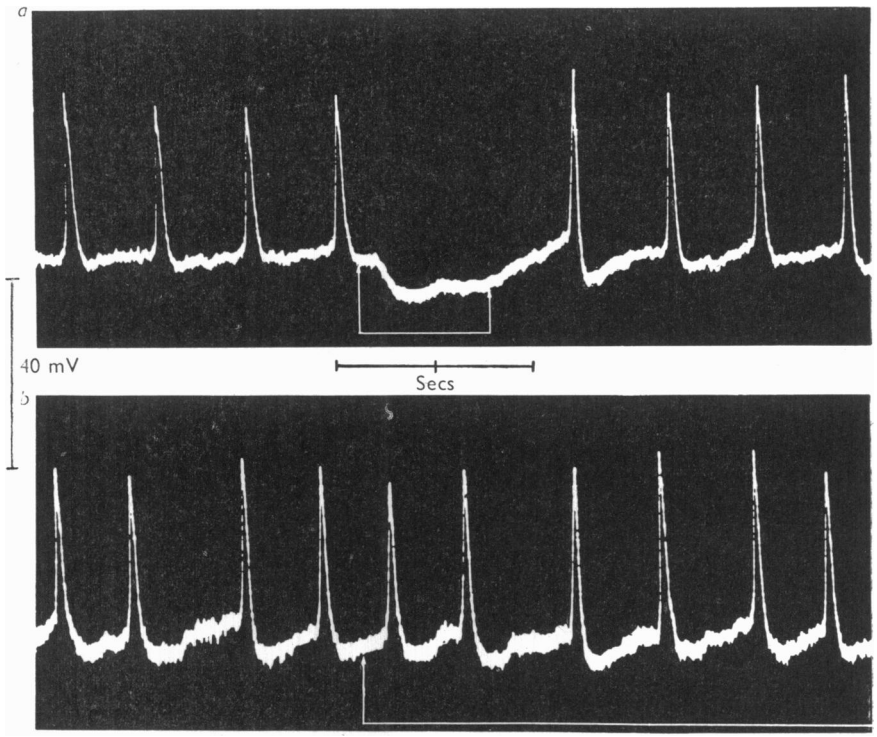

Fig. 7. The effect of bretylium (5×10^{-6} g/ml.) on the hyperpolarization due to stimulation of the perivascular inhibitory nerves. (*a*) Effect of stimulation of the perivascular nerves at 60 pulses/sec on the membrane potential of cell 55. (*b*) Effect of stimulation of these nerves at 60 pulses/sec on the membrane potential of cell 56, 30 min after the addition of bretylium. Period of stimulation indicated by the arrows. Pulse duration 200 μsec.

The effect of sympathetic blocking drugs on perivascular inhibition. Sympathetic blocking drugs abolished the hyperpolarizations due to perivascular inhibitory nerve stimulation. For example, in one preparation hyperpolarizations of up to 10 mV were recorded when the nerves were stimulated at 60 pulses/sec, as shown in Fig. 7*a*. Thirty minutes after the addition of bretylium (5×10^{-6} g/ml.) to the bath, there was no hyper-

polarization or change in the frequency of spontaneous activity when the perivascular nerves were stimulated, as shown in Fig. 7*b*.

Guanethidine (10^{-6} g/ml.) also blocked the hyperpolarization due to perivascular inhibitory nerve stimulation. The perivascular inhibitory nerves therefore behave as typical post-ganglionic sympathetic nerves.

DISCUSSION

Junction potentials have now been observed in response to single stimuli at all excitatory nerve-smooth muscle junctions so far examined (Burnstock & Holman, 1961; Ursillo, 1961; Gillespie, 1962*b*; Orlov, 1962; Speden, 1964). However, apart from the responses of the taenia coli to intramural inhibitory nerve stimulation (Bennett, Burnstock & Holman, 1966), no junction potentials have been observed in response to single stimuli at the two other inhibitory nerve-smooth muscle junctions so far examined, i.e. the transmission from sympathetic nerves to the distal colon (Gillespie, 1962*a*) and from the perivascular inhibitory nerves to the taenia coli. The probable reason for this difference between excitatory and inhibitory transmission is that in the latter case the concentration of transmitter reaching the muscle cells after a single stimulus is not sufficient to produce a detectable change in the membrane potential. The concentration of transmitter at a muscle cell is probably dependent on the amount of transmitter released by the nerves, the number of nerve fibres innervating the muscle cell, the distance of these nerves from the muscle cell and the rate of inactivation of the transmitter. Any combination of these factors could be responsible for the difference between the two modes of transmission. In the case of sympathetic inhibitory transmission the concentration of transmitter at the muscle cells must be increased by repetitive stimulation at frequencies from 5 pulses/sec to 10 pulses/sec before there is a detectable change in the membrane potential.

The latency for transmission of inhibition from perivascular inhibitory nerves, as large as 270 msec, is much greater than that recorded for transmission from excitatory junctions. Speden (1964) recorded a latency as great as 175 msec for transmission from sympathetic nerves to mesenteric blood vessels, while Kuriyama (1963) has recorded latencies as small as 6 msec for transmission from the hypogastric nerves to the vas deferens. Three main factors contribute to the latency in addition to the time for conduction along the nerves: the time for liberation of the transmitter from the nerves; the time for diffusion of transmitter from the nerve to the receptor sites and the time for the transmitter to produce the permeability changes at the post-synaptic membrane responsible for the post-synaptic potential changes (Eccles, 1964). The long latencies recorded for peri-

vascular transmission may be due to any three of these factors. However, it is interesting that a long diffusional distance between the inhibitory nerves and the smooth muscle could explain both why there are no junctional potentials in response to single pulses and why there is such a long latency for transmission. An electron-microscope study of the relation between the autonomic nerves and the smoooth muscle cells of the taenia coli is at present being made.

There was an increase in the amplitude and rate of rise of the hyperpolarization due to increasing frequencies of perivascular stimulation. An explanation of these changes may be found in a study of the potential changes at the motor end-plate due to electrophoretic injection of ACh made by Del Castello & Katz (1955). They showed that as the amount of ACh released at a point near the end-plate was increased, there was an increase in the rate of rise and magnitude of the potential change at the end-plate. Thus, if the amount of transmitter at the smooth muscle cells is increased by increasing the frequency of stimulation of the perivaseular nerves, then it might be expected that the amplitude and rate of rise of the hyperpolarization should be increased.

Small hyperpolarizations of the cell membrane in response to perivascular nerve stimulation at low frequencies exposed small depolarizations of the membrane which occurred when an action potential would normally have been expected. Since these depolarizations had the same time course as the foot of the action potential it is possible that they represent electrotonic potentials which are responsible for the conduction of action potentials in this tissue. Furshpan (1964) has recently summarized the evidence that electrical coupling may occur across junctions where there is a fusion of opposing cell membranes. Recently such tight junctions have been observed between intestinal smooth muscle cells by Dewey & Barr (1962) and by Oosaki & Ishii (1964). Bennett & Rogers (personal communication) have observed such tight junctions between apposing smooth muscle cells of the taenia coli and Bülbring, Burnstock & Holman (1958) have shown electrical activity in one smooth muscle cell of the taenia coli which was coincident with activity in an adjacent cell. It is probable that the small depolarizations exposed by hyperpolarizing the smooth muscle cells which were not showing pacemaker-like activity are due to electrical coupling from adjacent cells.

The decrease of spontaneous activity with an increase of the frequency of stimulation may explain why Burnstock *et al.* (1965) observed an increase in the relaxation of the taenia coli when there was an increase in the frequency of stimulation of the perivascular nerves. Since Bülbring & Kuriyama (1963) have shown that the tension developed by the taenia coli is related to the frequency of firing of the action potentials, a progressive

decrease in this firing due to increasing the frequency of stimulation of the nerves will give an increase in the relaxation of the muscle.

The time for the interval between action potentials to return to normal after perivascular nerve stimulation increased with increasing frequency of stimulation. This effect may explain why Burnstock *et al.* (1965) observed that the taenia coli continues to relax for some time after perivascular nerve stimulation has ended. Since the tension developed by the taenia is related to the frequency of firing of the action potentials, a decrease of the normal action potential firing rate after stimulation will probably maintain the muscle in a relaxed state. This delay in recovery suggests a slow rate of inactivation of transmitter.

The hyperpolarization due to perivascular nerve stimulation showed many similarities to the membrane potential changes produced in these cells by the direct application of adrenaline (Bülbring, 1957; Burnstock, 1958).

Since the hyperpolarization did not occur in the presence of sympathetic blocking drugs and the hyperpolarization is so like that observed by Gillespie (1962*a*) when stimulating the sympathetic nerves to the distal colon, the perivascular inhibitory nerves are probably post-ganglionic and sympathetic nerves.

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