J. Physiol. (1981), **316**, *pp.* 369–378
With 7 text-figures
Printed in Great Britain

ATROPINE-RESISTANT DEPOLARIZATION IN THE GUINEA-PIG SMALL INTESTINE

BY R. A. R. BYWATER, M. E. HOLMAN AND G. S. TAYLOR
From the Neuropharmacology Group, Department of Physiology, Monash University, Clayton, Victoria 3168, Australia

(*Received 6 November 1980*)

SUMMARY

1. Junction potentials were recorded from the circular muscle cells of the guinea-pig ileum following transmural stimulation in the presence of atropine at 30 °C.

2. Single stimuli produced a transient hyperpolarization, the inhibitory junction potential (i.j.p.). At high stimulus strengths the i.j.p. was followed by a post-stimulus depolarization (PSD).

3. During repetitive stimulation the magnitude of the hyperpolarization decreased; however, at the end of the stimulus period the PSD was enhanced and often reached threshold for the generation of action potentials. Thus, the size of the PSD was not directly related to the degree of the preceding hyperpolarization.

4. Hyperpolarization of the circular muscle cells was produced by the application of anodal current using large external electrodes. Rapid cessation of the applied current produced a transient after-depolarization which was shorter in time course than the PSD following the i.j.p. If the applied anodal current was reduced slowly (at a rate which mimicked the decrease in the hyperpolarization during repetitive nerve stimulation) no after-depolarization was observed.

5. Conditioning hyperpolarization of the circular muscle cells reduced the amplitude of the i.j.p. The i.j.p. was reversed at membrane potentials greater than approximately −90 mV.

6. The PSD did not appear to be due to the extracellular accumulation of potassium ions following the i.j.p. since the PSD persisted even when the i.j.p. was reversed.

7. The neurotoxin apamin reversibly abolished the i.j.p. and unmasked a transient excitatory junction potential (e.j.p.) with a variable latency (350–900 ms).

INTRODUCTION

When a segment of guinea-pig small intestine is stimulated with transmural electrodes at low frequencies (up to 10 Hz) for periods of 2–5 s, the circular layer may fail to contract during stimulation (Wood & Marsh, 1973) or contract only after a latency of about 2 s (Kottegoda, 1970). When the stimulus is turned off a secondary, often prolonged (5 s or more) contraction occurs. Although atropine abolishes contractions which occur during brief periods of stimulation at low frequencies, the post-stimulus contraction is potentiated by this compound (see Kottegoda, 1970; and

Fig. 4 of Wood & Marsh, 1973). Kottegoda interpreted his results as indicating that transmural stimulation activated inhibitory fibres to the muscle together with excitatory cholinergic fibres. He suggested that the atropine-resistant post-stimulus contraction was due to the release of an additional excitatory transmitter having 'greater stability, and therefore persistence whose effect breaks through when stimulation stops' (Kottegoda, 1970). On the other hand Wood and his colleagues considered that post-stimulus contraction was a myogenic 'rebound' phenomenon related to membrane depolarization following hyperpolarization of the circular muscle by non-adrenergic inhibitory nerves (Wood & Marsh, 1973). This interpretation could explain why atropine potentiated post-stimulus contraction, since the hyperpolarization during stimulation would be greater and rebound depolarization enhanced if simultaneous cholinergic excitation were reduced or abolished by the presence of atropine.

The present experiments, using intracellular techniques to record from circular smooth muscle cells of circular strips of guinea-pig small intestine, have been carried out in order to investigate further the cause of post-stimulus contraction. Preliminary accounts of some of these results have been reported elsewhere (Bernath, Bywater, Holman, Surprenant & Taylor, 1976; Bywater & Taylor, 1979).

METHODS

Guinea-pigs of either sex weighing 250–500 g were stunned, bled and a short segment (~3 cm) of ileum removed. The ileum was opened along the mesenteric border and a full-thickness strip (~15 mm × 2 mm) was cut parallel to the circular muscle fibres. The strips were mounted serosal surface uppermost in an organ bath as described by Abe & Tomita (1968), with approximately equal lengths of tissue being placed within the stimulating and recording compartments. The composition of the normal physiological solution and the details of the electrophysiological recording techniques have been described previously (Bywater & Taylor, 1980). Inhibitory junction potentials (i.j.p.s) were recorded with intracellular micro-electrodes placed less than 1 mm from one of the stimulating electrodes in response to rectangular current pulses (0·5 ms duration, various voltages). All experiments described in this report were carried out at 30 °C after an initial equilibration period of approximately 60 min at 37 °C. Atropine sulphate ($1{\cdot}4 \times 10^{-6}$ M) was present throughout all experiments.

RESULTS

Effects of single transmural stimuli

At 30 °C, in the presence of atropine ($1{\cdot}4 \times 10^{-6}$ M), circularly cut preparations showed little or no spontaneous activity and it was possible to advance the micro-electrode through the serosa and longitudinal muscle in order to impale the circular muscle. The membrane potential of the circular muscle ranged from 40 to 65 mV (forty preparations), in agreement with previously published data on this tissue (Suzuki & Kuriyama, 1975). No spontaneously occurring changes in membrane potential of either brief or long time course were observed. Following transmural stimulation (0·5 ms duration at 0·1Hz) i.j.p.s were recorded which could be graded in amplitude by varying the applied stimulus voltage. A series of i.j.p.s recorded during a continuous impalement in response to a progressive increase in stimulus strength is shown in Fig. 1. The latency from the onset of the stimulus artifact to 10% of the peak amplitude decreased from 250 ms (Fig. 1*A*) to 120 ms (Fig. 1*G*). Such i.j.p.s were not observed in the longitudinal muscle layer. Although guanethidine

was not used in these experiments, the low frequencies of stimulation make it unlikely that any effects would have been observed from the excitation of sympathetic nerves.

As the strength of the applied voltage was increased the repolarization phase of the i.j.p.s often 'overshot' the level of the resting membrane potential, producing transient post-stimulus depolarization (PSD). Although PSDs were associated with

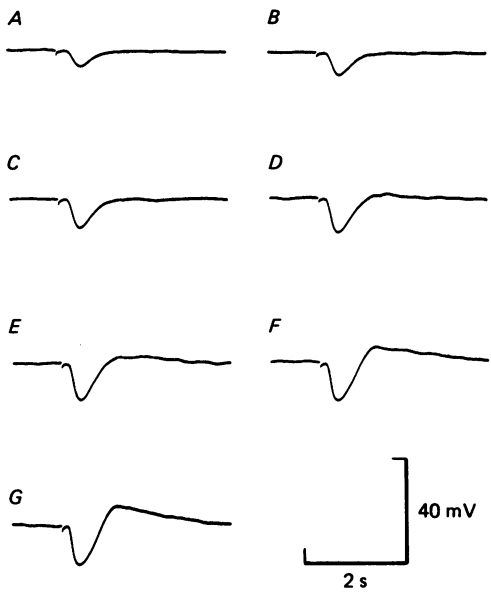

Fig. 1. The effects of increasing the stimulus strength, using single transmural stimuli of 0·5 ms duration, on the i.j.p. and PSD (*A*, 35 V; *B*, 40 V; *C*, 44 V; *D*, 48 V; *E*, 50 V; *F*, 55 V; *G*, 60 V). At higher stimulus voltages (*E*, *F*, and *G*) the decay of the i.j.p. continued beyond the level of the resting membrane potential producing PSD.

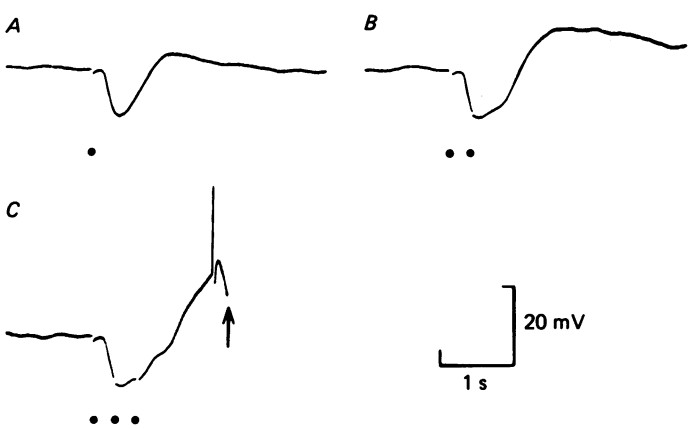

Fig. 2. The effects of one, two or three stimuli (3·5 Hz, 0·5 ms duration) on the amplitude of the PSD. *A*, a single stimulus produced a small PSD. *B*, following two stimuli the amplitude and duration of the PSD was increased even though the hyperpolarization following the second i.j.p. was reduced. *C*, following three stimuli the PSD reached threshold for action potential generation and the subsequent muscle contraction dislodged the micro-electrode (see arrow).

large stimulus strengths and thus large-amplitude i.j.p.s, in a few preparations large i.j.p.s were not always followed by PSD. The size of the PSD was therefore not directly related to the degree of the preceding hyperpolarization. This was more evident following repetitive stimulation (see below and Holman & Weinrich, 1975, and Bernath *et al.* 1976).

Effects of repetitive stimuli

The effects of increasing the number of stimuli from one to three (3·5 Hz) are shown in Fig. 2. Even though the amplitude of the hyperpolarization for each of

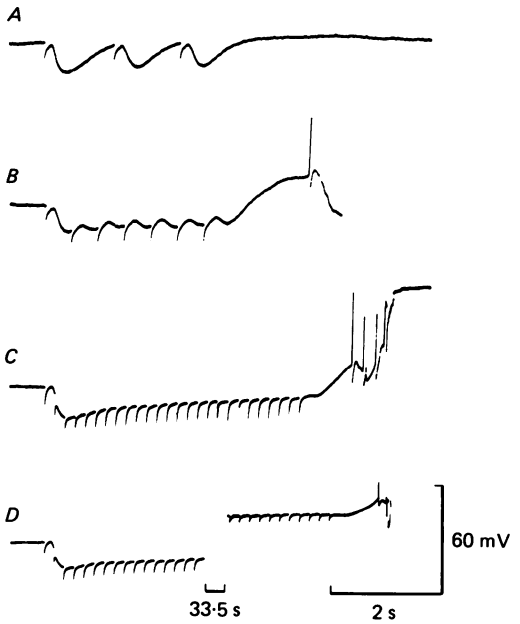

Fig. 3. The effects of repetitive stimulation on PSD. *A*, 0·75 Hz; *B*, 2 Hz; *C*, 5 Hz. *D*, stimulation at 5 Hz was maintained for 38·5 s; 33·5 s of the recording has been omitted, during which time the membrane potential slowly depolarized to approximately 18 mV above the resting membrane potential. In *A–D* the amplitude of the PSD was not related to the level of the immediately preceding hyperpolarization at the end of the train.

the successive stimuli decreased, the amplitude of the PSD was markedly increased (Fig. 2*B*, *C*). PSD was also evident following stimulus trains of different frequencies and was frequently associated with one or more action potentials which dislodged the micro-electrode (arrow in Fig. 2*C*). A series of responses to different frequencies of stimulation is shown in Fig. 3. The amplitude of the hyperpolarization from the resting potential, following the last stimulus of the train, decreased as the frequency of stimulation increased (Fig. 3*A* to Fig. 3*C*) but the PSD shown in Fig. 3*C* is larger than that shown in Fig. 3*A*. In Fig. 3*D* the duration of the stimulus train was prolonged (38·5 s) and at the end of this period the membrane potential was depolarized by 18 mV above the resting membrane potential. PSD still occurred after this train of stimuli at 5 Hz.

These observations support the suggestion above that the amplitude of PSD was not directly related to the degree of the preceding hyperpolarization.

Action potentials of varying amplitudes were recorded during PSD. This variation in amplitude may result from action potentials arising some distance from the recording micro-electrode and spreading passively to the recording site, or partial inactivation of the action-potential-generating mechanisms due to prolonged depolarization (see Fig. 3*D*), or partial dislodgement of the micro-electrode following movement of the tissue.

PSD and the depolarization following electrotonic potentials

Long-duration (700 ms) hyperpolarizing current pulses produced hyperpolarizing electrotonic potentials. As the amplitude of the electrotonic potentials was increased the decay was followed by a brief after-depolarization (Fig. 4*A*). The after-

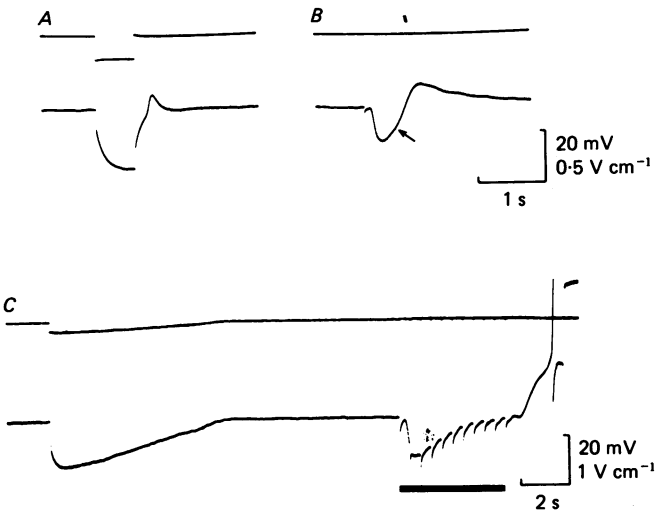

Fig. 4. Membrane potential changes during electrotonic potentials or transmural nerve stimulation. In each panel the upper trace is the applied field strength (V cm^{-1}), the lower trace the membrane potential (mV). *A*, hyperpolarizing electrotonic potential showing small transient after-depolarization upon rapid termination of current pulse. *B*, slow PSD following a single i.j.p. in response to nerve stimulation (0·5 ms duration, indicated by a dot) in the same cell as *A*. The arrow points to an inflexion during the decay phase of the i.j.p. which appears to represent the onset of the PSD response. *C*, hyperpolarizing electrotonic potential showing absence of after-depolarization following slow termination of the current pulse (left side of Figure); on the right side of the Figure is shown a decrease in hyperpolarization of a similar magnitude and time course during repetitive nerve stimulation (3 Hz, 0·5 ms, duration shown by bar) that is followed by PSD, an action potential, and dislodgement of the micro-electrode.

depolarization was not blocked by tetrodotoxin, whereas tetrodotoxin abolished both the i.j.p. and PSD. The recording in Fig. 4*B* shows an i.j.p. in response to transmural nerve stimulation. Although the amplitude of the electrotonic potential in the same cell (Fig. 4*A*) is greater than that of the i.j.p., the after-depolarization following the electrotonic potential is smaller in amplitude and briefer in time course than the PSD following nerve stimulation. Note also that in Fig. 4*B* the repolarization phase of the i.j.p. appears to consist of two components, i.e. at approximately 600 ms following the stimulus the rate of depolarization increases (see arrow in Fig. 4*B*), and is continuous with the rising phase of the PSD. Transient after-depolarizations were only observed following rapid termination of hyperpolarizing current pulses. Fig. 4*C*

shows the results of an experiment in which the electrotonic hyperpolarization was reduced slowly, at a rate similar to that observed during repetitive nerve stimulation. When the membrane potential was returned slowly to its resting level there was no after-depolarization. However, following repetitive nerve stimulation at 3 Hz in the same cell PSD reached threshold for action potential generation and the microelectrode was dislodged from the cell.

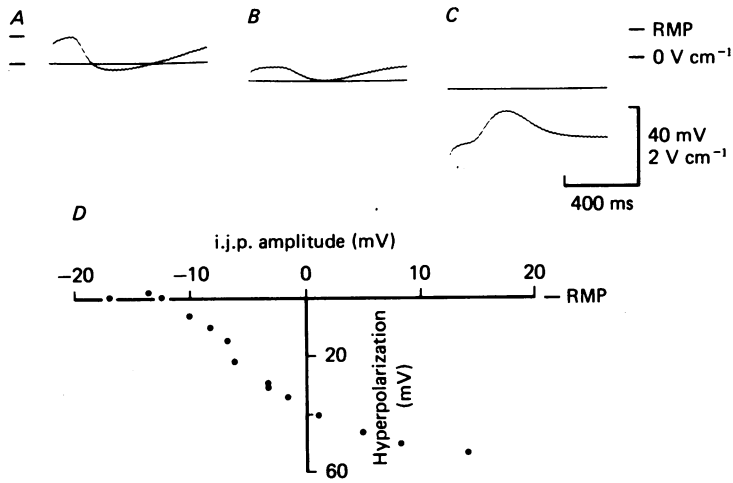

Fig. 5. The amplitude of the i.j.p. at different membrane potentials. At the extreme right and left of the Figure the level of the resting membrane potential (RMP) and the zero for the applied field strength (0 V cm^{-1}) is indicated for the three panels *A*, *B* and *C*. *A*, upper trace: single i.j.p. in response to transmural stimulation (0·5 ms duration) at RMP; lower trace: zero applied conditioning current. *B*, following conditioning hyperpolarization of approximately 14 mV the amplitude of the i.j.p. was reduced. *C*, following conditioning hyperpolarization of 53 mV the i.j.p. was reversed. *D*, the relationship between the amplitude of the i.j.p. (abscissa) and the magnitude of the conditioning hyperpolarization from the RMP (ordinate). The i.j.p. was reversed when the magnitude of the conditioning hyperpolarization was greater than 38 mV (interpolated intercept on ordinate).

Reversal potential of the i.j.p.

The amplitude of the i.j.p., following a single stimulus, depended upon the level of membrane potential. When the membrane potential was hyperpolarized the amplitude of the i.j.p. became successively smaller and was eventually reversed. Fig. 5 shows the results of an experiment in which i.j.p.s were evoked at different levels of applied hyperpolarizing current. In Fig. 5*B* the membrane potential was hyperpolarized by 14 mV and the i.j.p. was almost abolished. The i.j.p. was clearly reversed (Fig. 5*C*) when the membrane potential was hyperpolarized by 53 mV. The reversal potential for the i.j.p. was estimated from a plot of the amplitude of the i.j.p. *versus* the level of membrane hyperpolarization (Fig. 5*D*) to be about −90 mV. It was also observed that the time course of the inverted i.j.p. was considerably shorter than that of an i.j.p. evoked at the resting membrane potential. (Similar results were observed when the length of tissue in the recording chamber was less than one length constant.) The value of the membrane potential at which the i.j.p. became reversed is similar to the Nernst equilibrium potential for potassium ions (−89 mV) in the

guinea-pig taenia coli (Casteels, 1969). These observations suggest that in the circular muscle of the guinea-pig ileum the i.j.p. is caused by an increase in potassium conductance of the smooth muscle membrane. Similar observations have been reported for the guinea-pig taenia coli by Tomita (1972).

Is PSD caused by potassium ions?

In a further series of experiments we explored the possibility that the PSD was caused by an accumulation of potassium ions outside the smooth muscle cells following the increase in potassium conductance responsible for the i.j.p. When the membrane

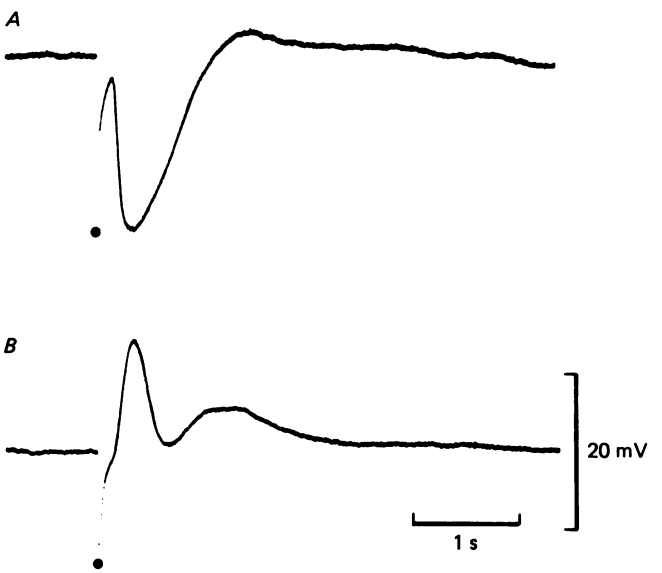

Fig. 6. PSD following the reversed i.j.p. *A*, control i.j.p. at the resting membrane potential; stimulus indicated by a dot. *B*, following conditioning hyperpolarization (67 mV) transmural stimulation (indicated by the dot) produced a reversed i.j.p. which was followed by PSD which had a latency of about 600 ms.

potential was hyperpolarized to −122 mV by prolonged anodal current to a level beyond the Nernst equilibrium potential for potassium ions the reversed i.j.p. was still followed by a depolarization as shown in Fig. 6. At this level of membrane potential the potassium flux resulting from the increase in potassium conductance during the i.j.p. would be inward; therefore the PSD is unlikely to be due to an extracellular accumulation of potassium ions.

The effects of apamin

A neurotoxic polypeptide extract of bee venom, apamin, has been shown to block the i.j.p. and relaxation during transmural stimulation in the guinea-pig taenia coli, caecum and the stomach (Vladimirova & Shuba, 1978; Banks, Brown, Burgess, Burnstock, Claret, Cocks & Jenkinson, 1979; Shuba & Vladimirova, 1980). In the circular muscle of the guinea-pig ileum, apamin (5×10^{-7} g/ml) blocked the i.j.p. and, as demonstrated for the taenia coil and caecum by Shuba & Vladimirova (1980), an atropine-resistant excitatory junction potential (e.j.p.) was revealed. Fig. 7 (*A* and

B) shows the hyperpolarizing response to transmural stimulation before apamin and an e.j.p. recorded after 20 min exposure to apamin. The effects of apamin were reversible after washing with apamin-free solution for about 40 min.

The latency for the atropine-resistant e.j.p. from four experiments ranged from 350 to 900 ms. Thus the latency for the e.j.p. is longer than for the control i.j.p., which rarely exceeded 250 ms (usually ~100 ms). The latency for the onset of the e.j.p. was

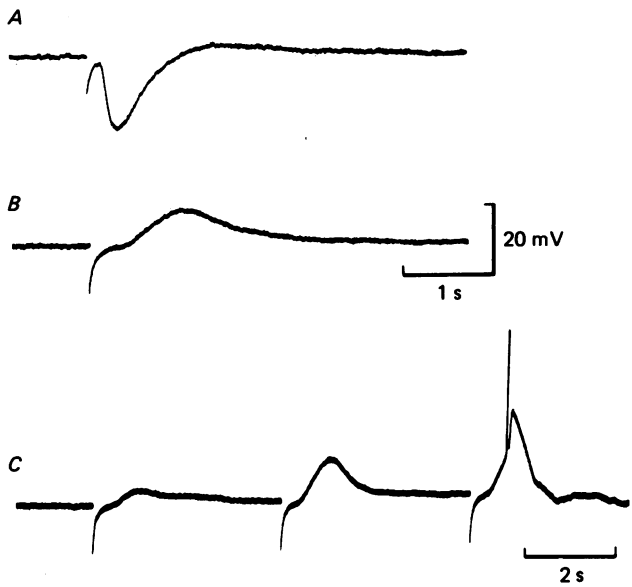

Fig. 7. The effects of apamin on the i.j.p. *A*, control i.j.p. *B*, the effects of a single transmural stimulus after 20 min exposure to 5×10^{-7} g/ml apamin: atropine-resistant e.j.p. with a latency of about 450 ms was observed. *C*, during repetitive stimulation (0·25 Hz) the e.j.p. showed facilitation and produced an action potential.

similar to the latency of the inflexion during the decay phase of the control i.j.p. (see also arrow in Fig. 4*B* and p. 373). A similar inflexion during the recovery phase of i.j.p.s was observed by Bennett, Burnstock & Holman (1966) in the guinea-pig taenia coli. During repetitive stimulation (0·25 Hz) the atropine-resistant e.j.p.s showed facilitation and reached threshold for an action potential (Fig. 7*C*).

DISCUSSION

It is clear that transmural stimulation of the circular layer of the guinea-pig small intestine in the presence of atropine causes a complex, long-latency depolarizing response (PSD) which can give rise to action potentials and contraction. The main aim of the present experiments was to determine whether the depolarization was a direct consequence of the hyperpolarization associated with i.j.p. We confirmed that the relatively rapid decay of membrane potential associated with the 'off' phase of a step function of hyperpolarizing current could cause a brief sub-threshold depolarization. However, when the changes in membrane potential associated with the repeated stimulation of inhibitory nerves were mimicked by hyperpolarizing current,

of a similar time course, no such depolarization occurred. We conclude that some other mechanism must be causing PSD.

Since the i.j.p. is associated with an increase in potassium conductance, it seemed possible that synchronous stimulation of intramural inhibitory nerves may have led to depolarization as a consequence of the accumulation of potassium ions in the narrow extracellular spaces between the tightly packed cells of this smooth muscle. We attempted to test this possibility by displacing the membrane potential to a level more negative than the potassium equilibrium potential, at which the polarity of the i.j.p. was reversed. We were still able to record PSD. On some occasions this was large enough to initiate action potentials and contractions. However, the relation between the amplitude of PSD and membrane potential is not simple and will be discussed in a future paper.

Vladimirova & Shuba (1978) and Shuba & Vladimirova (1980) have proposed that the same transmitter which causes the i.j.p. also acts on a second set of receptors to cause an atropine-resistant depolarization. They favour the hypothesis that the inhibitory transmitter is ATP, as suggested by Burnstock (1972). In their hands the taenia coli gave similar atropine-resistant responses to nerve stimulation and applied ATP and the hyperpolarizing action of the inhibitory transmitter and ATP were both blocked by the neurotoxin apamin (Shuba & Vladimirova, 1980). Burnstock, Cocks, Paddle & Staszewska-Barczak (1975) have reported that indomethacin (50 μM) abolished post-stimulus contraction in the guinea-pig taenia coli and similar observations were made by den Hertog & van den Akker (1979). The latter authors also stated that in the presence of indomethacin (50 μM) 'the rebound depolarization tended to decrease'. In contrast, Maas & den Hertog (1980) reported that although the phenyl phosphate prostaglandin antagonist N-0164 (10 μM) depressed the post-stimulus contraction in the guinea-pig taenia coli it did not alter post-stimulus depolarization. Furthermore, Shuba & Vladimirova (1980) have noted that in the presence of apamin, indomethacin (50 μM) failed to block the atropine-resistant e.j.p.s in this tissue. The role played by prostaglandins in post-stimulus excitatory phenomena is still uncertain. Preliminary experiments (M. E. Holman, unpublished work) suggested that indomethacin tended to increase the amplitude of post-stimulus contraction of circular strips of guinea-pig small intestine, but the electrophysiological basis of these observations has yet to be explored.

Our studies show that apamin also blocks the i.j.p. but not PSD in the circular muscle layer of guinea-pig small intestine. However, it should be emphasized that the latency of the atropine-resistant depolarization following transmural stimulation in the guinea-pig taenia coli and caecum (Shuba & Vladimirova, 1980) was much shorter than that observed in our experiments and we are therefore cautious in attributing PSD to the same transmitter as that which causes the i.j.p. Recently, Franco, Costa & Furness (1979*b*) have demonstrated in the guinea-pig ileum that following transmural stimulation hyoscine-resistant contractions were abolished by prior desensitization to substance P. Furthermore they have also demonstrated that a compound with substance-P-like activity is released following transmural stimulation (Franco, Costa & Furness, 1979*a*). Substance P or a similar compound may well be involved in the PSD responses reported in this paper.

In contrast to the atropine-resistant junction potentials following transmural

stimulation reported in this paper, all descending excitation following local balloon distension of the small intestine was abolished by atropine (Hirst, Holman & McKirdy, 1975). The role of atropine-resistant excitation in the neuronal control of intestinal motility therefore remains unclear.

This work was supported by a grant from the Australian Research Grants Committee. We are grateful to Dr B. Banks of University College London for supplying us with the apamin used in these experiments.

REFERENCES

ABE, Y. & TOMITA, T. (1968). Cable properties of smooth muscle. *J. Physiol.* **196**, 87–100.

BANKS, B. E. C., BROWN, C., BURGESS, G. M., BURNSTOCK, G., CLARET, M., COCKS, T. M. & JENKINSON, D. H. (1979). Apamin blocks certain neurotransmitter-induced increases in potassium permeability. *Nature, Lond.* **282**, 415–417.

BENNETT, M. R., BURNSTOCK, G. & HOLMAN, M. E. (1966). Transmission from intramural inhibitory nerves to the smooth muscle cells of the guinea-pig taenia coli *J. Physiol.* **182**, 541–558.

BERNATH, V. F., BYWATER, R. A. R., HOLMAN, M. E., SURPRENANT, A. & TAYLOR, G. S. (1976). Atropine resistant responses of the circular muscle of guinea-pig small intestine to repetitive nerve stimulation. *Proc. Aust. physiol. pharmacol. Soc.* **7**, 167*P*.

BURNSTOCK, G. (1972). Purinergic nerves. *Pharmac. Rev.* **24**, 509–581.

BURNSTOCK, G., COCKS, T., PADDLE, B. & STASZEWSKA-BARCZAK, J. (1975). Evidence that prostaglandin is responsible for the 'rebound contraction' following stimulation of non-adrenergic, non-cholinergic ('purinergic') inhibitory nerves. *Eur. J. Pharmac.* **31**, 360–362.

BYWATER, R. A. R. & TAYLOR, G. S. (1979). Atropine-resistant junction potentials in the guinea-pig small intestine. *Proc. Aust. Physiol. Pharmac. Soc.* **10**, 233*P*.

BYWATER, R. A. R. & TAYLOR, G. S. (1980). The passive membrane properties and excitatory junction potentials of the guinea-pig vas deferens. *J. Physiol.* **300**, 303–316.

CASTEELS, R. (1969). Calculation of the membrane potential in smooth muscle cells of the guinea-pig taenia coli by the Goldman equation. *J. Physiol.* **205**, 193–208.

DEN HERTOG, A. & VAN DEN AKKER, J. (1979). Effects of prostaglandin E_2 and indomethacin on the rebound of the guinea-pig taenia coli. *Eur. J. Pharmac.* **55**, 143–148.

FRANCO, R., COSTA, M. & FURNESS, J. B. (1979*a*). Evidence that axons containing substance P in the guinea-pig ileum are of intrinsic origin. *Naunyn-Schmiedebergs Arch. Pharmac.* **307**, 57–63.

FRANCO, R., COSTA, M. & FURNESS, J. B. (1979*b*). Evidence for the release of endogenous substance P from intestinal nerves. *Naunyn-Schmiedebergs Arch. Pharmac.* **307**, 195–201.

HIRST, G. D. S., HOLMAN, M. E. & MCKIRDY, H. C. (1975). Two descending nerve pathways activated by distension of guinea-pig small intestine. *J. Physiol.* **244**, 113–127.

HOLMAN, M. E. & WEINRICH, J. P. (1975). The effects of calcium and magnesium on inhibitory junctional transmission in smooth muscle. *Pflügers Arch.* **360**, 109–119.

KOTTEGODA, S. R. (1970). Peristalsis of the small intestine. In *Smooth Muscle*, ed. BÜLBRING, E., BRADING, A., JONES, A. & TOMITA, T, pp. 525–541. London: Edward Arnold.

MAAS, A. A. J. & DEN HERTOG, A. (1980). The effect of the phenyl phosphate N-0164 on prostaglandin action and on post inhibitory excitation in the taenia of guinea-pig caecum. *Eur. J. Pharmac.* **62**, 157–166.

SHUBA, M. F. & VLADIMIROVA, I. A. (1980). Effect of apamin on the electrical responses of smooth muscle to adenosine 5′-triphosphate and to non-adrenergic, non-cholinergic nerve stimulation. *Neuroscience* **5**, 853–859.

SUZUKI, H. & KURIYAMA, H. (1975). Electrical and mechanical properties of longitudinal and circular muscles of the guinea-pig ileum. *Jap. J. Physiol.* **25**, 759–773.

TOMITA, T. (1972). Conductance change during the inhibitory potential in the guinea-pig taenia coli. *J. Physiol.* **225**, 693–703.

VLADIMIROVA, I. A. & SHUBA, M. F. (1978). The effect of strychnine, hydrastin and apamin on synaptic transmission in smooth muscle cells. *Nierofiziologiya, Kiev* **10**, 295–299.

WOOD, J. D. & MARSH, D. R. (1973). Effects of atropine, tetrodotoxin and lidocaine on rebound excitation of guinea-pig small intestine. *J. Pharmac. exp. Ther.* **184**, 590–598.