Characteristic features of inhibitory junction potentials evoked by single stimuli in the guinea-pig isolated taenia caeci

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1. Changes in membrane potential of the guinea-pig isolated taenia caeci evoked by single stimuli have been investigated using intracellular recording techniques. Nifedipine (10 μM) was used to arrest spontaneous muscle action potentials. Single stimuli elicited complex junction potentials which consisted of both excitatory and inhibitory components.

2. The excitatory component of the compound junction potential was unaffected by hexamethonium (100 μM) but abolished by atropine (1 μM) and ω-conotoxin GVIA (10–100 nM).

3. In the presence of atropine, single stimuli elicited fast inhibitory junction potentials (IJPs). IJPs were sometimes biphasic during repolarization with a noticeable 'slow tail'. Apamin (30–100 nM) potently inhibited the fast IJP and revealed an underlying slow IJP.

4. The fast IJP was also abolished by ω-conotoxin GVIA (100 nM). However, the slow IJP was insensitive to ω-conotoxin GVIA but was abolished by cadmium (30 μM).

5. Guanethidine (3 μM) and $N\omega$-nitro-L-arginine (10–100 μM) had no detectable effects on either of the IJPs. The dye Reactive Blue 2 reduced the amplitude of the fast IJP but this reduction was associated with a membrane hyperpolarization.

6. The existence of two distinct IJPs in the guinea-pig taenia caeci has been demonstrated. The ability of ω-conotoxin GVIA to selectively abolish the fast IJP leaving the slow IJP intact suggests that separate nerves are involved in mediating these responses.

The taenia of the guinea-pig caecum has been widely used to study the mechanisms by which autonomic nerves control the activity of smooth muscle (Bennett, 1972). Early electrophysiological studies of the taenia caeci largely concentrated on postjunctional mechanisms and, in particular, how neurotransmitters modified the spontaneous firing of muscle action potentials (Burnstock, 1958; Bennett, 1972). The spontaneous firing of action potentials in smooth muscle has made a detailed analysis of neurotransmitter-induced changes in membrane potential complicated, since muscle contraction frequently dislodges intracellular microelectrodes. Consequently, the mechanisms governing the release of neurotransmitters producing junction potentials and the nature of the calcium channels regulating neurotransmitter release in the taenia have not been investigated in detail.

Surprisingly little is known about the precise identity of the neurotransmitters producing excitatory and inhibitory responses in the guinea-pig taenia caeci. A bewildering number of putative neurotransmitter candidates have been proposed, using immunohistochemical, electrophysiological and mechanical techniques (Bennett, Burnstock & Holman, 1966; Campbell, 1966; Burnstock, Campbell, Satchell & Smythe, 1970; Cocks & Burnstock, 1979; Satchell, 1981; Costa, Furness & Humphreys, 1986; Iselin, Martin, Magistretti & Ferrero, 1988; Furness, Pompolo, Shuttleworth & Burleigh, 1992; Knudsen & Tøttrup, 1992; McConalogue, Furness, Vremec, Holst, Tornøe & Marley, 1995). It is clear that there is a cholinergic excitatory (Bennett, 1966; Campbell, 1966) and a noradrenergic inhibitory (Bennett *et al.* 1966) innervation. However, there is still a degree of uncertainty as to the precise identity of the neurotransmitter mediating the inhibitory junction potential (IJP) and relaxation produced by electrical field stimulation of the intramural nerve fibres. It has been suggested that there is a purinergic inhibitory innervation in the guinea-pig taenia caeci (Burnstock *et al.* 1970; Mackenzie & Burnstock, 1980; Satchell, 1981) but there is

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also evidence that vasoactive intestinal polypeptide (VIP) (Fahrenkrug, 1979; Furness, Costa & Walsh, 1981; Iselin *et al.* 1988), nitric oxide (NO) (Knudsen & Tøttrup, 1992; Poitrowski, Simon & Brennan, 1993) and more recently, pituitary adenylyl cyclase activating peptide (PACAP) (Jin, Katsoulis, Schmidt & Grider, 1994; McConalogue *et al.* 1995) are inhibitory neurotransmitters.

The development of selective calcium and potassium channel blockers has allowed the innervation of the guinea-pig taenia caeci to be studied with a degree of resolution previously unobtainable, using electrophysiological techniques. In the present study, the L-type calcium channel antagonist nifedipine was used to abolish smooth muscle action potentials and therefore mechanical activity. Individual components of the excitatory and inhibitory junction potentials evoked by electrical field stimulation were isolated using various physiological and pharmacological procedures. In particular, the nature of the calcium channels controlling the release of the inhibitory neurotransmitter(s) was investigated.

METHODS

Preparation

Male Dunkin–Hartley guinea-pigs (200–350 g) were killed by a blow to the head, bled, and the taenia dissected from the caecum. As far as possible, all circular smooth muscle was removed from the underside of the taenia longitudinal muscle. Strips of taenia *circa* 2·0 cm in length were pinned out to approximately 1·5 times their resting length, on the base of a Sylgard (Dow Corning)-covered 2 ml Perspex organ bath with the aid of fine-gauge tungsten wire pins (0·5 mm diameter). The preparation was transilluminated and visualized using a Leica binocular operating microscope (magnification ×6 to ×40). The preparation was superfused at a rate of 2 ml min^{-1} with warmed (37 °C) Krebs solution of the following composition (mM): NaCl, 120; KCl, 5·9; $NaHCO_3$, 15·4; $MgCl_2$, 1·5; NaH_2PO_4, 1; $CaCl_2$, 2·5; and glucose, 11; gassed with 97% O_2 and 3% CO_2 to pH 7·4.

Intrinsic nerves were activated by field stimulation using a pair of Ag–AgCl electrodes arranged in parallel about 3 mm apart. The tissue was transversely orientated with one end of the muscle strip woven between the stimulating electrodes. Single stimuli (pulse width, 0·04–0·5 ms; 30 V) were delivered from a digital stimulator (Applegarth Electronics, Oxford, UK). In all preparations, nifedipine (10 μM) was added to the perfusing Krebs solution to abolish smooth muscle action potentials. Drugs were made up as concentrated stock solutions in distilled water (ethanol, in the case of nifedipine and atropine) and serially diluted in Krebs solution to the required final bath concentration. Drugs were applied by changing the Krebs solution perfusing the preparation to one containing the appropriate drug.

Electrophysiology

Changes in membrane potential of individual smooth muscle cells were measured using conventional intracellular recording techniques. Filamented microelectrodes (external diameter, 1·5 mm) were backfilled with 0·5 M KCl, and had resistances of 70–150 MΩ. In all preparations, penetrations were made about 1 cm from the stimulating electrodes to facilitate comparisons between preparations.

Collection and analysis of data

Spontaneous and evoked electrical activity was recorded on-line using an Axoclamp II (Axon Instruments Inc., Foster City, CA, USA) coupled to a Lab Master DMA 12-bit A/D converter (Scientific Solutions Incorporated, OH, USA) installed in an IBM-compatible 486 PC. Signals were digitized at 500 Hz and collected using Axotape (version 1.2; Axon Instruments Inc.).

The latency, amplitude and time courses of electrical signals were determined using Axotape (IBM PC compatible) and Kaleidagraph (version 3.0; Synergy Software, Reading, PA, USA) running on an Apple Macintosh using exported ASCII files.

Drug solutions

The following drugs were used: apamin, Basilen Blue E-3G (Reactive Blue 2), guanethidine monosulphate, hexamethonium chloride, nifedipine, *N*ω-nitro-L-arginine and tetrodotoxin (Sigma Chemical Co., Poole, UK), atropine and cadmium chloride (BDH Chemicals, Poole, UK), ω-conotoxin GVIA (Alomone Labs, Israel).

Statistics

All data are expressed as means ± S.E.M., where n refers to the number of cells. Data were tested for statistical significance using Student's unpaired t test. Values of $P < 0·05$ were considered to be statistically significant.

RESULTS

Spontaneous activity

In the absence of nifedipine, action potentials were recorded in most smooth muscle cells and these were usually associated with spontaneous contractions of the muscle. When nifedipine (10 μM) was added to the perfusion solution, the muscle action potentials and associated contractions were rapidly abolished. Under these conditions, the mean resting membrane potential of smooth muscle cells was $-41 \pm 0·5$ mV ($n = 29$ cells in 8 preparations). The use of nifedipine facilitated the study of junction potentials since it was now possible to carry out single cell experiments before and after drug treatments.

Activity evoked by a single stimulus in the presence of nifedipine

Field stimulation of the intrinsic nerve fibres, with a single stimulus, produced a complex junction potential with both excitatory and inhibitory components. The stimulus strength was varied by increasing the pulse width in steps from 0·04 to 0·5 ms, keeping the voltage constant (30 V). While the precise nature of the evoked electrical activity could vary from cell to cell and from preparation to preparation, in general, at stimulus durations below 0·1 ms, IJPs predominated. However, when the pulse width was increased, junction potentials with both excitatory and inhibitory components could be evoked. The excitatory component of the responses was graded with stimulus strength reaching a maximum at a pulse width of 0·3–0·5 ms (Fig. 1). The individual components of these complex electrical responses were isolated using pharmacological agents.

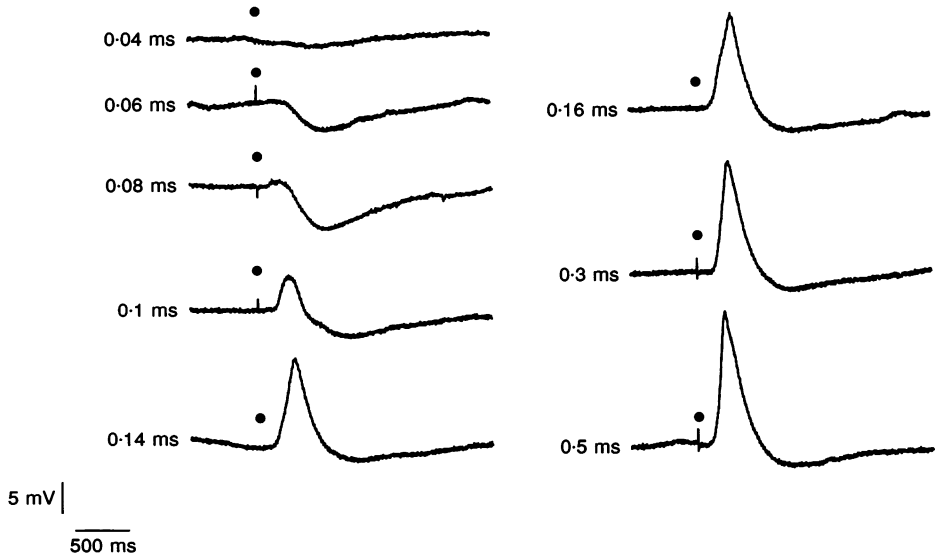

Figure 1. Examples of junction potential complexes evoked by field stimulation of the guinea-pig taenia caeci

Junction potentials were evoked by single stimuli (●; pulse widths, 0·04–0·5 ms; 30 V) in the presence of nifedipine (10 μM). When the stimulus duration was varied, negative-going or positive–negative-going junction potentials were recorded which reflect the changes in membrane potential produced by the concomitant release of excitatory and inhibitory neurotransmitters. The resting membrane potential of the smooth muscle cells ranged between −41 and −43 mV. The presence or absence of a stimulus artifact on some traces reflects the rapid time course of the stimulus artifact compared with the A/D sampling rate used to digitize junction potentials.

Effects of atropine and hexamethonium

When the muscarinic receptor antagonist atropine (1 μM) was added to the perfusion solution, the excitatory component of the multiphasic junction potential was abolished. Under the conditions of these experiments, only hyperpolarizations were evoked when the stimulus duration was varied (Fig. 2). It would appear that the major excitatory junction potential (EJP) was evoked by a single stimulus cholinergic in origin and mediated by an action of acetylcholine on muscarinic receptors, confirming the results of Bennett (1966). Hexamethonium (100 μM) had no effect on the EJP, showing that postganglionic nerve fibres were being activated. To investigate the properties of IJPs alone, all subsequent experiments, except where indicated, were carried out in the presence of atropine (1 μM).

The inhibitory junction potential has two components

In the presence of atropine (1 μM) and nifedipine, single stimuli evoked IJPs which were graded with stimulus

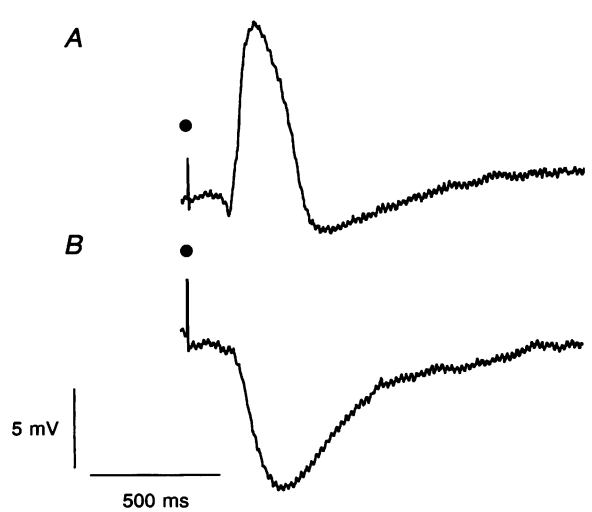

Figure 2. Effect of atropine on the excitatory component of the junction potential complex

A, positive–negative-going junction potential evoked by a single stimulus (●; 0·3 ms, 30 V) in the presence of nifedipine (10 μM). *B*, atropine (1 μM) inhibited the excitatory component of the complex to reveal an IJP. The resting membrane potential of the smooth muscle cells ranged between −41 and −43 mV.

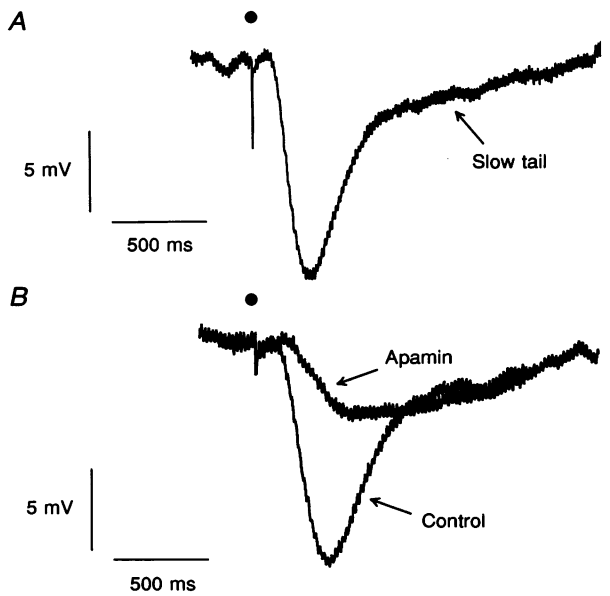

Figure 3. Effect of apamin on the fast component of the inhibitory junction potential

A, IJP evoked by a single stimulus (●; 0·3 ms, 30 V) in the guinea-pig taenia caeci in the presence of nifedipine (10 μM) and atropine (1 μM) displaying both the initial fast hyperpolarization and the slow tail observed during the recovery phase. *B*, apamin (100 nM) abolishes the fast component of the IJP to reveal a slow IJP with a longer latency. The resting membrane potential of the smooth muscle cells ranged between −41 and −43 mV.

strength (0·04–0·5 ms, 30 V). In general, a pulse width of 0·3 ms at 30 V produced a maximal hyperpolarization and these parameters were used for the rest of the experiments. IJPs evoked by single stimuli had a latency of 144 ± 4 ms ($n = 12$ cells in 8 preparations), and a mean amplitude of 15 ± 1 mV ($n = 12$ cells in 8 preparations). Sometimes the IJP appeared biphasic in time course, the rapid repolarization phase being interrupted by a slow 'tail' which prolonged the time course (Fig. 3*A*). The mean duration of the IJP (at 90% decay) was about 1711 ± 179 ms ($n = 13$ cells in 6 preparations). The presence of a tail, which tended to be more pronounced in some preparations than others, suggested that more than one conductance and/or a second neurotransmitter was involved in the generation of the IJP. These components were investigated by using apamin to block small-conductance calcium-activated potassium channels and ω-conotoxin GVIA, to block N-type calcium channels.

Effects of apamin on the inhibitory junction potential

When apamin (30 or 100 nM), was added to the perfusion solution, the initial fast component of the IJP was rapidly abolished (2–4 min) and a second, slower delayed IJP was revealed (Fig. 3*B*). The characteristics of the slow IJP differed from that of the apamin-sensitive IJP, having a longer latency (231 ± 9 ms, $n = 8$ cells in 8 preparations), a smaller amplitude (5·4 ± 0·5 mV, $n = 8$ cells in 8 preparations) and a prolonged time course (see below and Fig. 6). Thus, it would appear that two inhibitory transmitters are responsible for producing the IJP complex. Interestingly, in some preparations, particularly following apamin treatment, occasional small atropine-resistant EJPs were evoked together with the slow IJP (Fig. 4) but these will not be considered further in the present inquiry.

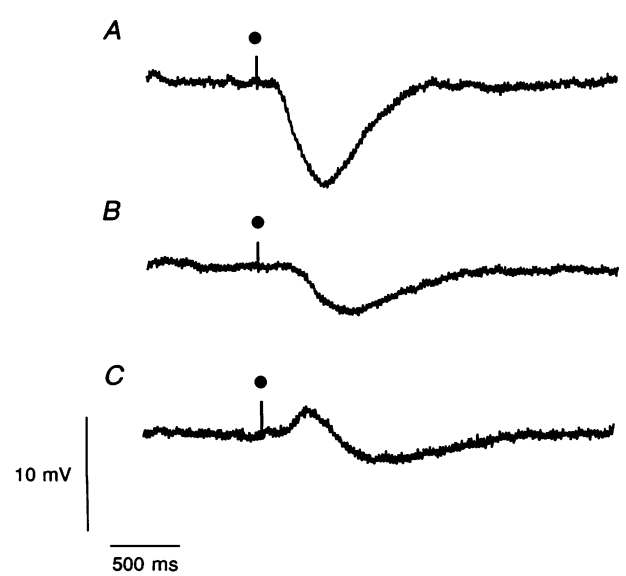

Figure 4. Effect of apamin on junction potentials evoked by a single stimulus in the presence of atropine and nifedipine: occurrence of an atropine-resistant excitatory junction potential

A, IJP evoked by a single stimulus (●; 0·3 ms, 30 V) in the guinea-pig taenia caeci in the presence of atropine (1 μM) and nifedipine (10 μM). *B*, apamin (30 nM) abolishes the fast IJP. *C*, example of an atropine-resistant EJP recorded in the same cell at the same stimulus strength after apamin had been present for 10 min. The resting membrane potential of the smooth muscle cells ranged between −41 and −43 mV.

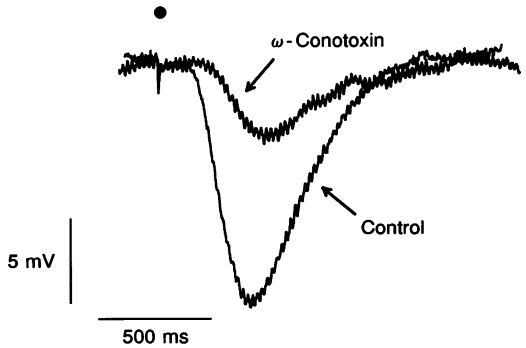

Figure 5. Effect of ω-conotoxin GVIA on the fast component of the inhibitory junction potential
IJP evoked by a single stimulus (●; 0·3 ms, 30 V) in the guinea-pig taenia caeci in the presence of nifedipine (10 μM) and atropine (1 μM). When ω-conotoxin GVIA (100 nM) was added to the perfusion fluid, the initial rapid component of the IJP was abolished revealing an underlying IJP at a longer latency. The resting membrane potential of the smooth muscle cells ranged between −41 and −43 mV.

Effects of ω-conotoxin GVIA

The second IJP could also be isolated using ω-conotoxin GVIA. After the application of ω-conotoxin GVIA (10–100 nM) for approximately 15 min, the initial fast IJP was abolished but the slow IJP remained (Fig. 5). The slow IJP had the same characteristics as the IJP remaining in the presence of apamin with a latency of 218 ± 14·5 ms and an amplitude of 5·3 ± 0·3 mV ($n = 6$ cells in 6 preparations). Indeed, there was no significant difference in the latency and amplitude of the slow IJPs isolated by either apamin or ω-conotoxin GVIA ($P = 0·45$ and 0·95, respectively), although the difference in latency between the fast and the slow IJPs was highly significant ($P < 0·0001$). These findings suggest that the two inhibitory transmitters mediating the fast and the slow IJP are coming from separate nerves and that the calcium channel regulating the release of the inhibitory transmitter responsible for the slow IJP is not of the N-type.

By subtracting the time courses of the fast and slow IJPs (after treating cells with either apamin or ω-conotoxin GVIA to isolate the slow IJP) it was possible to get a better estimate of the true duration of the fast IJP. Using this method the fast IJP decayed to 50% amplitude in 442 ± 8·5 ms and the slow IJP in 909 ± 135 ms ($n = 4$ cells in 4 preparations). A representative plot of the subtracted time course in a single cell experiment is shown in Fig. 6.

In the continued presence of nifedipine, the combination of apamin alone and apamin in the presence of ω-conotoxin GVIA also provided further insights into neuroeffector transmission. An example of a compound excitatory and inhibitory junction potential complex, recorded in the absence of atropine, is shown in Fig. 7*A*. When apamin (30 nM) was applied to the preparation, the EJP became more prominent, showing that the initial fast IJP normally short circuits the EJP (Fig. 7*B*). It is noteworthy that from preparation to preparation and from cell to cell, the EJP was only present in about 15% of trials. The subsequent application of ω-conotoxin GVIA (10 nM), in the continued presence of apamin, abolished the prominent muscarinic EJP after about 15 min and clearly revealed the ω-conotoxin GVIA 'resistant' slow IJP (Fig. 7*C*). These results strongly suggest that the calcium channels controlling the release of acetylcholine and the neurotransmitter responsible for the fast IJP are of the N-type.

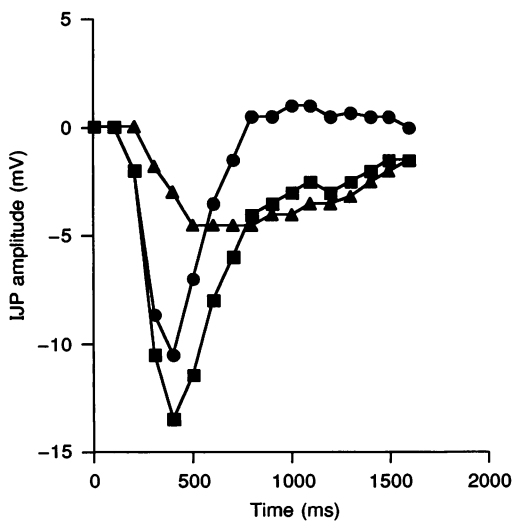

Figure 6. Time course of the fast and slow inhibitory junction potentials subtracted
When the time course of a slow IJP is subtracted from that of the combined IJP an estimation can be made of the approximate time course of a fast IJP in isolation. This graph shows the time course of both the fast and slow IJP from a typical cell in the taenia caeci. The slow IJP was isolated with apamin (30 nM). ▲, slow IJP; ■, fast IJP; ●, subtracted IJP.

Given the insensitivity of the slow IJP to both apamin and ω-conotoxin GVIA, it was important to establish whether the slow IJP was neuronal in origin. Tetrodotoxin (3 μM), which blocks voltage-dependent sodium channels, rapidly abolished both the fast and the slow IJPs (Fig. 8*A*).

Effects of cadmium on the slow inhibitory junction potential

The ineffectiveness of ω-conotoxin GVIA and of nifedipine to block the slow IJP, suggests that N- and L-type calcium channels are not involved in the release of the neurotransmitter responsible for the slow IJP. However, perfusion of the bathing solution with the non-selective calcium channel blocker cadmium chloride, (30 μM) abolished the slow IJP (Fig. 8*B*) and this effect was reversible on washing. The nature of the calcium channel controlling the release of the transmitter generating the slow IJP remains to be elucidated, using the wide range of specific calcium channel toxins which are becoming available.

Possible neurotransmitter candidates generating the inhibitory junction potential

In preliminary experiments, the effects of a number of antagonists were investigated in an attempt to identify the neurotransmitters responsible for mediating the IJPs. Given that the taenia receives a noradrenergic inhibitory innervation (Bennett *et al.* 1966), the adrenergic neurone blocker guanethidine was added to the perfusion solution. After a combination of guanethidine (3 μM) and atropine (1 μM) for more than 1 h, both IJPs could still be elicited by a single stimulus, demonstrating that neither of the responses were mediated by noradrenaline. Both IJPs are therefore mediated by non-adrenergic, non-cholinergic nerves.

It has been suggested that the neurotransmitter generating the IJP (although we now know there are two IJPs) in the guinea-pig taenia is ATP and that it mediates its effect via an action on purinoceptors of the P_{2y} subclass (Kennedy, 1990). Reactive Blue 2, a putative P_{2y} purinoceptor antagonist, was therefore used to determine if the IJP was mediated by this mechanism. Reactive Blue 2 (100 μM) reduced the amplitude of the IJP by 46 ± 11% ($n = 5$ cells in 5 preparations). However this inhibitory effect was associated with a membrane hyperpolarization of up to 20 mV (Fig. 9*A*). The relationship between maximum reduction in IJP amplitude and the degree of hyperpolarization produced by Reactive Blue 2 (100 μM) is plotted in Fig. 9*B*. There appeared to be a relationship between the maximum reduction in IJP amplitude and the degree of membrane hyperpolarization but the correlation coefficient barely failed to reach statistical significance. The correlation coefficient of the regression of the maximum reduction in IJP amplitude *versus* maximal membrane hyperpolarization was 0·745;

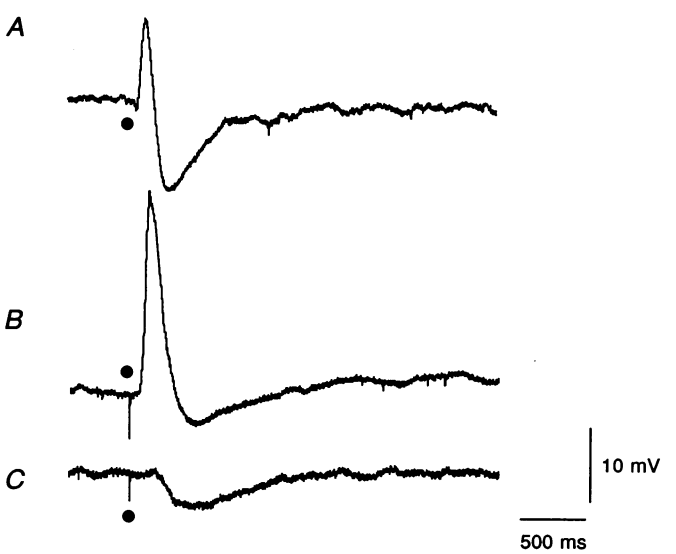

Figure 7. Effects of apamin and ω-conotoxin GVIA on junction potential complexes evoked by field stimulation

Junction potentials were evoked by single stimuli (●; 0·3 ms, 30 V) in the presence of nifedipine (10 μM). *A*, control. *B*, 15 min after apamin (30 nM). The record shows that the muscarinic EJP is normally short circuited by the fast IJP. *C*, 30 min after apamin and ω-conotoxin GVIA (10 nM) the muscarinic EJP is abolished showing that N-type calcium channels control the release of acetylcholine. An underlying IJP at a longer latency which is not blocked by ω-conotoxin GVIA is revealed. The resting membrane potential of the smooth muscle cells ranged between −41 and −43 mV.

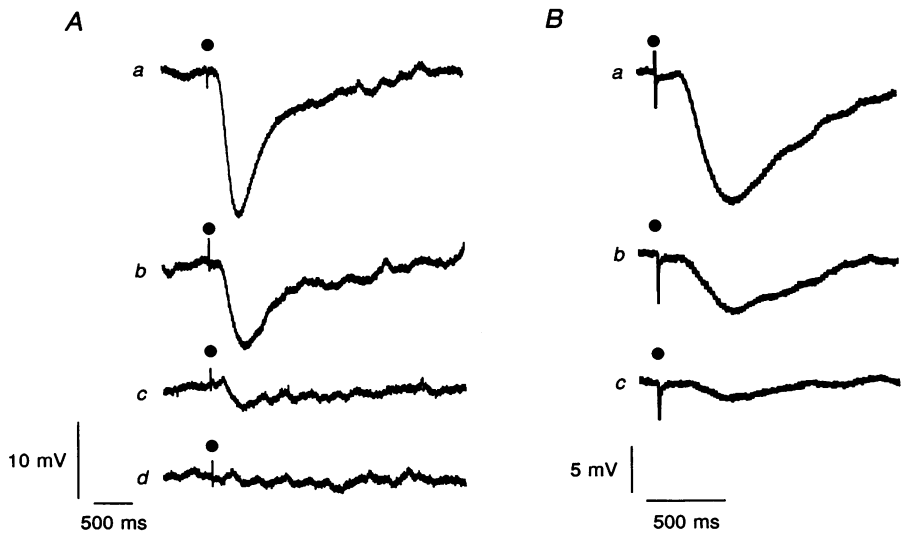

Figure 8. Effects of tetrodotoxin and cadmium on the inhibitory junction potential

A: *a*, IJP evoked by a single stimulus (●; 0·3 ms, 30 V) in the guinea-pig taenia caeci in the presence of nifedipine (10 μM) and atropine (1 μM); *b–d*, progressive effects of tetrodotoxin (3 μM) after 45, 65 and 90 s, respectively. *B*: IJPs evoked by single stimuli (●; 0·3 ms, 30 V) in the presence of nifedipine (10 μM) and atropine (1 μM); *a*, control; *b*, 15 min after ω-conotoxin GVIA (30 nM); *c*, 6 min after the concomitant addition of cadmium (30 μM). The fast but not the slow IJP was abolished by ω-conotoxin GVIA. The slow IJP was readily blocked by cadmium. Ten minutes after the perfusion of cadmium was started the slow IJP was abolished. The inhibitory effects of cadmium but not ω-conotoxin GVIA were reversed by washing. The resting membrane potential of the smooth muscle cells ranged between −41 and −43 mV.

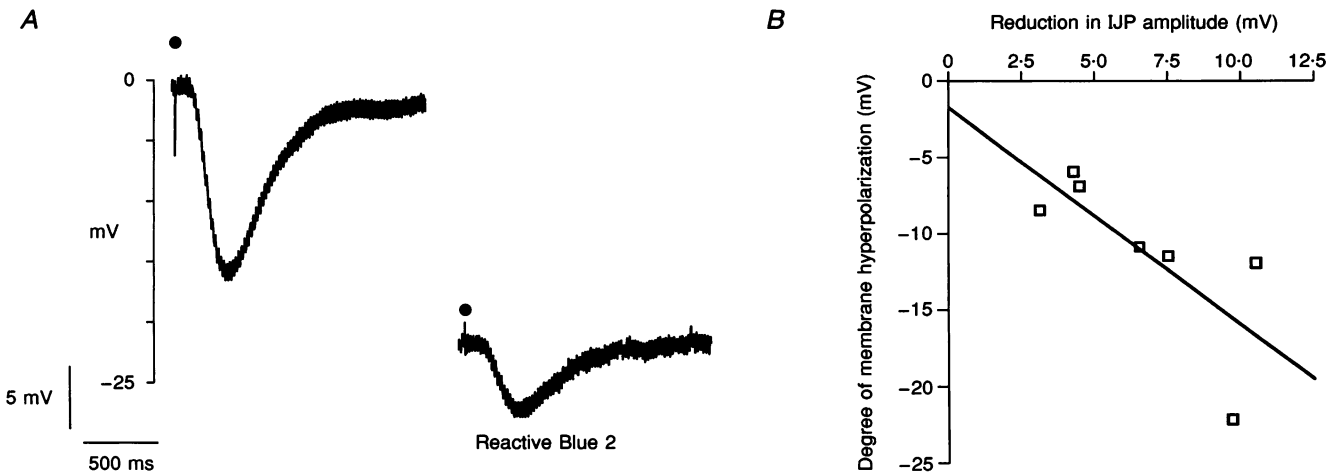

Figure 9. Effect of Reactive Blue 2 on the fast inhibitory junction potential

A, IJPs evoked by single stimuli (●; 0·3 ms, 30 V) in the presence of nifedipine (10 μM) and atropine (1 μM). The resting membrane potential of the smooth muscle cells ranged between −41 and −43 mV. Following Reactive Blue 2 treatment (100 μM) the membrane potential was seen to hyperpolarize and this was accompanied by a reduction in the amplitude of the IJP. *B*, plot of the degree of membrane hyperpolarization *versus* the reduction in IJP amplitude. Each point (□) represents the maximum reduction in IJP amplitude at the point of maximum membrane hyperpolarization, in 7 separate single cell experiments. The line of best fit gives a correlation coefficient of $r = 0{\cdot}745$ ($0{\cdot}05 < P < 0{\cdot}1$).

degrees of freedom, 5; $0{\cdot}05 < P < 0{\cdot}1$. The P_2 purinoceptor antagonist suramin has previously been shown to be effective in reducing IJP amplitude in the guinea-pig taenia (Den Hertog, Van den Akker & Nelemans, 1989) although more recent data would dispute this finding (McConalogue, Lyster & Furness, 1994).

Nitric oxide has been demonstrated to be a novel non-adrenergic, non-cholinergic (NANC) transmitter in a number of tissues in the gastrointestinal tract, and indeed mechanical studies have demonstrated a nitrergic relaxation in the guinea-pig taenia (Knudsen & Tøttrup, 1992; Poitrowski *et al.* 1993). The nitric oxide synthase inhibitor *N*ω-nitro-L-arginine (10–100 μM) was used to determine whether the IJPs were mediated by nitric oxide. However, IJPs were not detectably altered by the nitric oxide synthase inhibitor at concentrations shown to be effective in the taenia (Poitrowski *et al.* 1993) ($n = 5$ cells in 5 preparations).

DISCUSSION

The use of nifedipine to block L-type calcium channels and arrest spontaneous contractile activity has facilitated the examination of inhibitory transmission in the taenia of the guinea-pig caecum. With the tissue stretched to approximately 1·5 times its resting length, the mean resting membrane potential of the smooth muscle cells was *circa* −41 mV. This value is comparable with the −43 mV reported by Bülbring (1954) in her early classical microelectrode experiments. Bülbring described how the membrane potential was dependent on the degree of 'stretch' of the tissue and commonly, at the *in situ* length, the membrane potential of cells was of the order of −60 mV. Clearly, in the present experiments, nifedipine had little effect on the resting membrane potential of the smooth muscle cells at their 'stretched' tension even though the tissue was quiescent.

Under these conditions, when the intramural nerves of the taenia were stimulated with single pulses, complex electrical responses were evoked. Depending on the stimulus strength, either IJPs alone or an EJP–IJP complex was evoked. Since the EJPs tended to be elicited by stimuli of longer duration, it is likely that nerves of smaller diameter carry the excitatory transmitter. EJPs were elicited by stimulation of postganglionic cholinergic nerves since they were abolished by atropine and unaffected by hexamethonium. No attempt has been made to determine the nature of the muscarinic receptor subtype mediating the EJP. These findings are in agreement with the results of Bennett (1966), who showed that excitatory transmission from intramural nerves to smooth muscle cells of the guinea-pig taenia caeci could be blocked by atropine. In his paper Bennett noted the 'sparse' excitatory innervation of the taenia caeci, remarking on how IJPs predominated following nerve stimulation. In the present study, EJPs could only be evoked in about 15% of trials; even when the stimulus duration was varied over a wide range, in the majority of cases only IJPs were elicited. Often, EJP–IJP complexes were evoked and it was difficult to measure accurately the latency, amplitude and time course of individual junction potentials. When atropine was added, the EJPs were abolished and the magnitude of the IJP increased. Similarly, when the fast IJP was blocked with apamin, in those cells showing a positive–negative-going junction potential complex, the excitatory component was enhanced (see Fig. 7*B*). Thus, there is a complex interplay between excitatory and inhibitory junction potentials when they are elicited by field stimulation with single stimuli. When trains of stimuli are used to elicit IJPs in the presence of atropine, the repolarization phase is followed by an 'after-depolarization' which corresponds to a 'rebound contraction' in mechanical studies (M. Bridgewater & T. C. Cunnane, unpublished observation). However, 'after-depolarizations' are not usually recorded following an IJP elicited by a single stimulus.

Tomita (1972) reported that an increase in potassium permeability was the main factor responsible for the hyperpolarization of the cell membrane during an IJP. The ability of apamin (a polypeptide constituent of bee venom) to block the IJP suggests that the channels involved are the small-conductance calcium-activated K^+ channels. Despite the well-documented use of apamin to block IJPs in the taenia (Maas & Den Hertog, 1979; Maas, Den Hertog, Ras & Van den Akker, 1980; Shuba & Vladimirova, 1980; Maas, 1981) there has been no prior description of a second slow IJP. The closest description comes from Shuba & Vladimirova (1980) who described how apamin caused an increase in the latency of the IJP and a reduction in its amplitude. However, in their experiments, the IJP remaining in the presence of apamin was masked by the occurrence of a non-cholinergic EJP. It is noteworthy that, in the present investigation, atropine-resistant EJPs were recorded in some cells following apamin treatment, a phenomenon which deserves further investigation. Thus it seems probable that a second excitatory neurotransmitter produces non-cholinergic EJPs in the taenia, in response to a single stimulus. Pharmacological manipulation of the EJP–IJP complex has revealed at least four separate components; two excitatory and two inhibitory. The characteristic features of the junction potentials elicited in the guinea-pig taenia can be compared with those listed for a variety of different tissues in a review by Brock & Cunnane (1992).

It is noteworthy that the cholinergic EJPs evoked in the presence of apamin were blocked by low concentrations of ω-conotoxin GVIA, which irreversibly blocks N-type calcium channels. N-type calcium channels therefore

control the release of acetylcholine from postganglionic excitatory nerves. The fast IJP could be blocked postjunctionally by apamin, and prejunctionally by ω-conotoxin GVIA. The sensitivity of the fast IJP to ω-conotoxin GVIA suggests that N-type calcium channels also control the release of the inhibitory transmitter which generates the fast IJP. Conversely, the lack of effect of ω-conotoxin GVIA on the slow IJP indicates that N-type calcium channels do not control the release of the inhibitory transmitter mediating the slow IJP. It seems likely that the two inhibitory transmitters are released from separate nerves although it is conceivable that one nerve controls the release of two neurotransmitters through different populations of calcium channels in the secretory terminals. Further evidence in support of the former conclusion comes from the action of tetrodotoxin which tended to abolish the fast IJP before the slow IJP. Equally, when the stimulus duration was varied, occasionally the slow IJP could be evoked selectively. For example in Fig. 1, at a pulse width of 0·06 ms, only the slow IJP with a latency in excess of 200 ms was evoked, yet in Fig. 2, following atropine treatment, when a pulse width of 0·3 ms was employed, the fast IJP predominated. The fact that the two IJPs have a distinct difference in latency would also suggest that separate nerves mediate the two responses, although the presence of different postjunctional transduction mechanisms for the two neurotransmitters could account for this temporal difference. Indeed, it is possible that a single neurotransmitter is released that is acting through two types of postjunctional receptor. Despite its resistance to N- and L-type calcium channel blockers, the slow IJP was abolished by a non-selective calcium channel blocker, cadmium. A cautious interpretation is that cadmium could be acting postjunctionally to block calcium channels in the smooth muscle, which in turn could mediate the hyperpolarization; however, this seems unlikely at the modest concentrations used in these experiments (30 μM).

Preliminary attempts were made to establish the identity of the neurotransmitters generating the IJPs by examining the effects of several antagonists. Bennett *et al.* (1966) have demonstrated that the guinea-pig taenia caeci receives a noradrenergic inhibitory innervation. However, guanethidine had no detectable effect on either of the IJPs, suggesting that noradrenergic nerves are not involved. Classically, ATP has been proposed as the mediator of the fast IJP in the taenia (Burnstock *et al.* 1970; Mackenzie & Burnstock, 1980; Satchell, 1981), through an action on receptors of the P_{2y} subtype (Kennedy, 1990). When the P_{2y} purinoceptor antagonist Reactive Blue 2 (Kennedy, 1990) was applied to the tissue, the fast IJP was reduced in amplitude. Fast IJPs elicited in the rat caecum have also been shown to be antagonized by Reactive Blue 2 (Manzini, Hoyle & Burnstock, 1986). It is possible, therefore, that ATP mediates one of the IJPs in the taenia. However, the reduction in amplitude of the fast IJP was associated with cell membrane hyperpolarization and this hyperpolarization *per se* may have produced the inhibition, rather than P_{2y} purinoceptor antagonism. In order to determine whether the reduction in IJP amplitude was due to purinoceptor antagonism, it would be necessary to compare the rate of change of IJP amplitude with respect to the change in membrane potential induced by Reactive Blue 2 and current, respectively (Tomita, 1972). Interestingly, Manzini *et al.* (1986) reported a 3 mV depolarizing effect of Reactive Blue 2 in addition to its reduction of IJP amplitude in the rat caecum. However, they used the Reactive Blue 2 isomer known as Cibacron Blue 3GA which has an A-ring *o*-sulphonic acid as opposed to the *m*- or *p*-sulphonic acid in the A-ring of Basilen Blue E-3G (see Sigma catalogue). The discrepancy in the results between the present study and those of Manzini *et al.* (1986) may therefore be due to the use of a different isomer of this dye and deserves investigation. Further evidence in support of a purinergic IJP are the reports that the non-selective P_2 purinoceptor antagonist suramin antagonizes mechanical responses to P_2 purinoceptor agonists and purinergic nerve stimulation in the guinea-pig taenia (Hoyle, Knight & Burnstock, 1990). In addition, it has been shown to reduce IJP amplitude (Den Hertog *et al.* 1989). However, more recent results would tend to dispute this finding (McConalogue *et al.* 1994). Clearly, until selective purinoceptor antagonists become available, the evidence that ATP is an inhibitory neurotransmitter in the guinea-pig taenia caeci should be accepted with caution.

Nitric oxide is involved in NANC transmission in a number of tissues throughout the gastrointestinal tract (Boeckxstaens, Pelckmans, Bult, De Man, Herman & Van Maercke, 1990; Toda, Baba & Okamura, 1990; Dalziel, Thornbury, Ward & Sanders, 1991; Stark, Bauer & Szurszewski, 1991; Maggi & Giuliani, 1993). It produces smooth muscle relaxation through the activation of guanylate cyclase and the subsequent elevation of cGMP levels (Arnold, Mittal, Katsuki & Murad, 1977; Shikano, Long, Ohlstein & Berkowitz, 1988; Kanada *et al.* 1992). In some tissues, nitrergic IJPs have been recorded (Dalziel *et al.* 1991; Ward, McKeen & Sanders, 1992). A nitrergic inhibitory innervation to the guinea-pig taenia has been demonstrated by immunohistochemical (Furness *et al.* 1992) and classical pharmacological (Knudsen & Tøttrup, 1992; Maggi & Giuliani, 1993; Poitrowski *et al.* 1993) techniques. However, the nitric oxide synthase inhibitor *N*ω-nitro-L-arginine, at concentrations which have previously been shown to prevent nitrergic transmission in the guinea-pig taenia (Poitrowski *et al.* 1993), had no detectable effects on either the fast or slow IJP. Clearly,

the relaxant effect of nitrergic nerve stimulation in guinea-pig taenia caeci is independent of changes in membrane potential, a phenomenon which has also been reported in studies of the rat proximal colon (Suthamnatpong, Hosokawa, Takeuchi, Hata & Takewaki, 1994).

In conclusion, we have shown that stimulation of the intramural nerves of guinea-pig taenia caeci with single stimuli can produce four distinct types of junction potential; two inhibitory and two excitatory. The two EJPs are produced by acetylcholine and an as yet unidentified NANC neurotransmitter. The identity of the neurotransmitters responsible for the two IJPs remains to be elucidated, although in the present experiments we have ruled out an involvement of nitrergic and noradrenergic nerves. We have also demonstrated that the calcium channels regulating the release of acetylcholine, and the neurotransmitter responsible for the fast but not the slow IJP, are of the N-type. The nature of the calcium channels controlling the release of the neurotransmitter responsible for the slow IJP remains unknown. The controversy surrounding the exact identity of the inhibitory transmitter may be explained in the light of the present experiments, given that at least two distinct IJPs exist. The ability of ω-conotoxin GVIA to isolate one IJP should facilitate further studies in inhibitory transmission in this preparation.

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