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ELECTRICAL AND MECHANICAL ACTIVITY RECORDED FROM RABBIT URINARY BLADDER IN RESPONSE TO NERVE STIMULATION

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SUMMARY

1. Responses of the smooth muscle membrane of the rabbit bladder to intramuscular nerve stimulation were investigated by the micro-electrode and double sucrose-gap methods.

2. The cell generated regular spontaneous action potentials. Acetylcholine produced a maintained increase in the frequency and ATP a transient increase. Noradrenaline only increased the frequency at very high concentrations.

3. Application of short current pulses (50 μsec) produced an initial excitatory junction potential (e.j.p.) with a superimposed spike, followed by a late depolarization. On some occasions, hyperpolarization of the membrane appeared between initial e.j.p. and the late depolarization. All these responses were abolished by tetrodotoxin.

4. The late depolarization was enhanced by pre-treatment with neostigmine and abolished by atropine. This means that the delayed depolarization is due to activation of the muscarinic receptor. When the late depolarization was abolished, the amplitude of hyperpolarization was enhanced.

5. The e.j.p. and contraction were unaffected by guanethidine, phentolamine, methysergide, mepyramine, quinidine or theophylline. This means that the e.j.p. is not mediated by activation of adrenergic, tryptaminergic, histaminergic or purinergic receptors.

6. ATP reduced the amplitude of the e.j.p. due to depolarization of the membrane and reduction in the membrane resistance. The amplitude of the e.j.p. was gradually reduced by repetitive stimulation (0·5–2·0 Hz). However, the rate of depression was unchanged in the presence of ATP. Dipyridamole did not change the electrical and mechanical responses to field stimulation. These results do not support the proposal that ATP is the non-cholinergic excitatory transmitter.

7. Apamine and tetraethylammonium (TEA) suppressed the hyperpolarization produced by field stimulation but guanethidine did not inhibit the hyperpolarization. Therefore, the hyperpolarization is due to increased K conductance of the membrane but it is not possible to conclude whether this component is due to the inhibitory action of a neurotransmitter or solely to after hyperpolarization of the spike.

8. It was concluded that the rabbit bladder receives both cholinergic and non-cholinergic excitatory neurones.

* Authors' names are in alphabetical order.

INTRODUCTION

The contractile response of the urinary bladder to parasympathetic (pelvic) nerve stimulation is only partially antagonized by atropine (Langley & Anderson, 1895; Ursillo & Clark, 1956) and is unaffected by phentolamine (Ambache & Zar, 1970; Creed & Tulloch, 1978). The response to sympathetic (hypogastric) nerve stimulation is also relatively insensitive to atropine or phentolamine and close arterial injection of catecholamines produces only a small rise in bladder pressure (Dave & Dhattiwala, 1976; Creed, 1979). Although it is possible that the receptor sites in the bladder are not readily accessible to exogenously applied drugs (Ursillo & Clark, 1956), it is now generally accepted that both nerves contain non-cholinergic, non-adrenergic fibres. However, the nature of the transmitter remains unsolved (Ambache, 1955; Taira, 1972).

Comparison of contractile responses to field stimulation and to various drugs has recently led to the proposal that there are excitatory purinergic nerves in the bladder which release ATP (Burnstock, Dumsday & Smythe, 1972; Burnstock, Cocks, Crowe & Kasakov, 1978; Dean & Downi, 1978). However, the lack of effect of dipyridamole, which is thought to inhibit uptake of the metabolite, adenosine (Dean & Downie, 1978) and the persistence of responses to field stimulation following tachyphylaxis to ATP (Ambache & Zar, 1970) do not support this proposal.

In the present experiments, electrical events in the smooth muscle cells of the rabbit bladder have been recorded. Firstly, the membrane properties were investigated with micro-electrodes. The responses of the tissue to field stimulation were then studied in the double sucrose-gap and the effects of specific antagonists on junctional potentials assessed. An early excitatory junction potential with a surperimposed spike and a late muscarinic depolarization were identified.

METHODS

Rabbits of either sex weighing between 2 and 3 kg were killed by a blow on the head, and bled. The urinary bladder was removed and after opening it by two lateral incisions the mucous membrane was removed.

For recording with micro-electrodes longitudinal strips 20 by 2 mm running from the dome of the bladder to the urethra were dissected and mounted with the serosal side uppermost in a bath similar to that described by Abe & Tomita (1968). The tissue was superfused with Krebs solution made hypertonic by addition of 15 g sucrose to 100 ml Krebs solution to abolish movement at a temperature of 32 °C. The electrical properties of the smooth muscle cells were recorded with glass micro-electrodes filled with 3 M-KCl and photographed from a cathode ray oscilloscope and also displayed on a pen recorder (RJG 4022 Nihon Kohden). The tissue was stimulated by application of current between two silver plates 10 mm apart through which one end of the tissue passed. Drugs were added either directly to the bath or to the perfusing fluid.

For double sucrose-gap recording, longitudinal strips 20 by 2 mm were cut from level of the ureter openings to the dome of the bladder along the line of the superficial smooth muscle bundles. The technique was similar to that described by Ito & Tajima (1981) with 0·5 mm of the middle of the strip in contact with Krebs solution. The two adjacent chambers contained isotonic sucrose. Electrical activity recorded between the central chamber and an outer chamber containing isotonic KCl was displayed on a pen recorder. Mechanical activity was also displayed via a force transducer. Field stimulation with pulses of 50 μsec was applied to a ring electrode in the centre chamber through which the tissue passed. Drugs were added to the Krebs solution perfusing the central chamber.

For mechanical recording muscle strips 8 by 1·5 mm were mounted in a 1·4 ml organ bath through which Krebs solution flowed continuously at 5 ml/min. The strips were set up with an initial tension of 0·2 g and mechanical activity was recorded via a force transducer on a pen recorder. Supramaximal field stimulation was applied through two ring electrodes round the tissue at 20 Hz (300 μsec pulse duration) and drugs were either added to the perfusing fluid or directly to the bath.

The Krebs solution had the following composition (mM): Na^+, 137·4; K^+, 5·9; Ca^{2+}, 2·6; Mg^{2+}, 1·2; Cl^-, 134·0; $H_2CO_3^-$, 15·5; HPO_4^-, 1·2; glucose, 11·5. The solution was equilibrated with 3 % CO_2 in O_2.

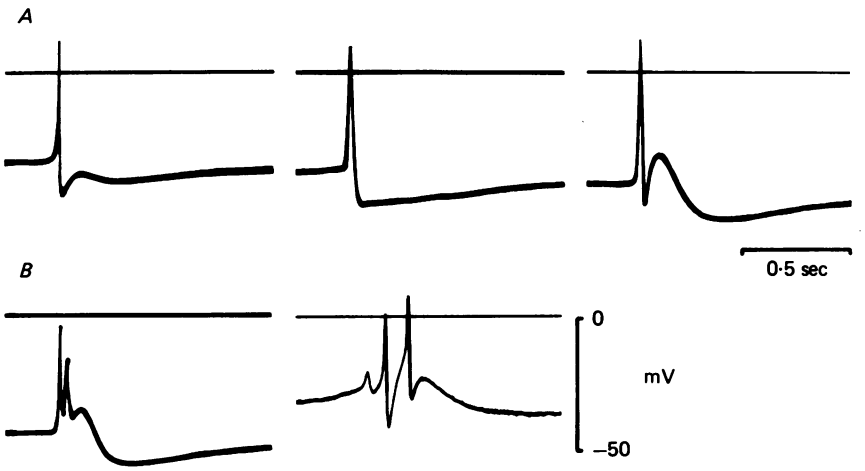

Fig. 1. Spontaneous action potentials recorded with intracellular micro-electrodes from rabbit urinary bladder. *A*, single spikes in a female rabbit from a cell in the trigone (left), between the ureter openings (middle) and in the detrusor muscle. *B*, multiple spikes from the detrusor muscle of two male rabbits. The records were photographed from an oscilloscope. The upper trace is zero potential and the lower trace the membrane potential.

RESULTS

General features of electrical properties

Dorsal strips were set up so that micro-electrodes could be inserted into cells between the dome of the bladder and the urethra up to 10 mm caudal to the ureter openings. The mean resting membrane potential for all cells was $-37·4 \pm 3·5$ mV ($n = 106$) (mean $\pm$ S.D.) and was similar in all regions and for strips from males or females. In most preparations set up for more than an hour, all parts had spontaneous activity though this often ceased after about 6 hr. The activity was in the form of simple or mulitiple action potentials which occurred regularly at 5–30 per minute. They tended to be more frequent towards the urethra.

Spikes recorded from all regions had overshoots of up to 18 mV but showed considerable variation in shape. In strips taken from females, spikes were usually single and arose abruptly from the resting potential. In some cells from both the bladder and urethra, this initial spike was immediately followed by hyperpolarization and a slower depolarization, so that recovery occurred only after more than 500 msec (Fig. 1*A*). In other cells, especially those near the ureter openings, the slow depolarization was absent and the hyperpolarization was prolonged. A range of intermediate shapes was also seen. Such action potentials were also seen in males but

multiple action potentials with two or more spikes superimposed on a depolarization also frequently occurred and often a depolarization preceded the spike (Fig. 1 *B*). The amplitude of the spikes was usually less than for the single spikes though the resting potential was within the same range.

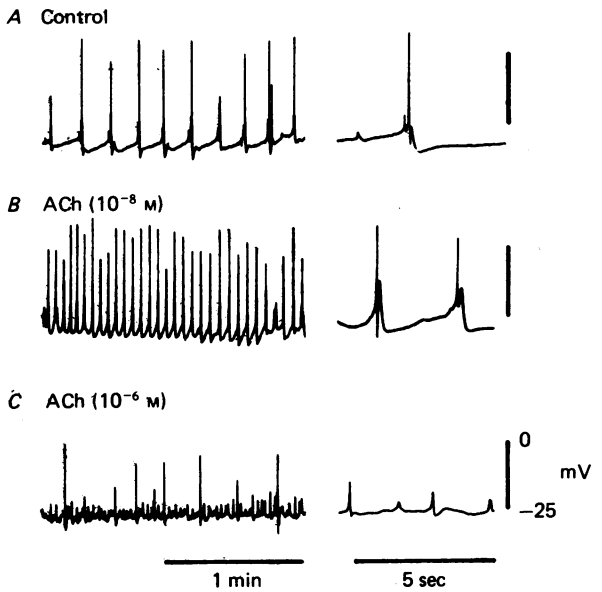

Fig. 2. The effect of acetylcholine (ACh) on spontaneous activity of the detrusor muscle recorded with a micro-electrode. The records were taken from a pen recording. At 10^{-8} M, ACh increased the frequency of action potentials and produced slight depolarization (*B*). 10^{-6} M produced further depolarization and asynchronous spike activity (*C*).

Acetylcholine (10^{-8} M) produced slight depolarization of the membrane with an increase in the frequency of action potentials (Fig. 2). At higher concentrations (10^{-6} M) a more marked depolarization occurred with asynchronous spike activity. Although noradrenaline increased spike frequency in the urethra at 10^{-6} M, it had no effect on the detrusor muscle at this concentration. At 10^{-4} M there was an increase in frequency with slight depolarization. Phenylephrine (10^{-4} M) mimicked this response, whereas isoprenaline (10^{-5} M) slowed or abolished spikes in the detrusor muscle. ATP (10^{-3} M) either had no effect or produced a transient increase in spike activity ($n = 6$).

In order to study the spread of activity within the tissue, square pulses were applied through extracellular plate electrodes and recorded at various distances from the stimulating plate. In the cranial part of the bladder (detrusor muscle), hyperpolarizing pulses of 1 sec duration produced an electrotonic potential which decayed exponentially with distance with a length constant (λ) varying from 1·85 to 2·61 mm (mean = 2·15 mm, $n = 6$). Depolarizing currents of 10 msec duration evoked spikes and these spread through the tissue at a conduction velocity of over 100 mm/sec. In the urethra, where the orientation of the smooth muscle bundles is less apparent, electrotonic potentials could not be recorded and depolarizing pulses either failed to evoke action potentials or they occurred in the impaled cell at varying intervals after

stimulation. The observations on the bladder suggest that the double sucrose-gap method is applicable for investigating membrane properties and neuromuscular transmission if the gap is shorter than 0·5 mm.

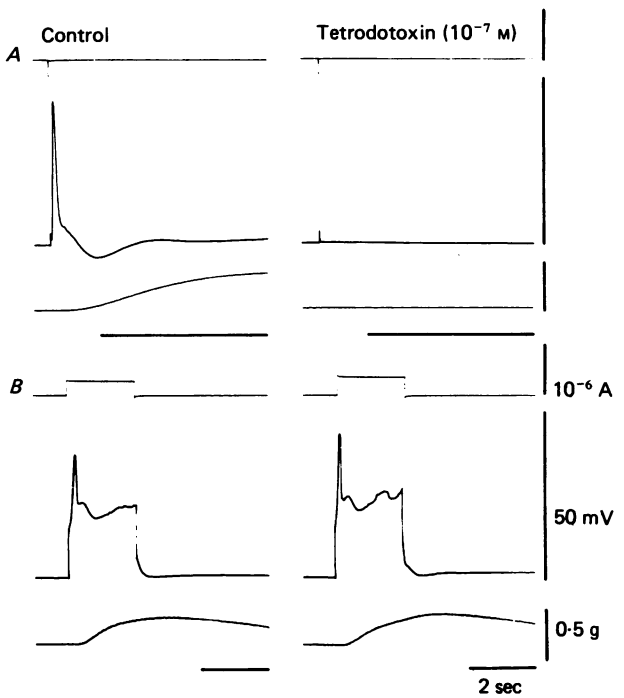

Fig. 3. The effect of tetrodotoxin on responses to stimulation of a strip from rabbit bladder recorded in the double sucrose-gap. *A*, field stimulation with pulses of 50 μsec duration produced a spike which was abolished by tetrodotoxin. *B*, spikes produced by depolarizing pulses of 2 sec duration were resistant to tetrodotoxin. The top trace indicates the stimulus, the middle trace is the electrical response and the bottom trace the mechanical response.

Electrical and mechanical activity recorded by the double sucrose-gap

In order to investigate the effects of field stimulation on the electrical and mechanical properties of smooth muscle cells of the rabbit bladder, we used the double sucrose-gap method. When preparations were first set up there were normally rhythmic contractions preceded by electrical activity. This ceased within 30 min in most preparations but occasionally continued throughout an experiment. In the presence of TEA (10^{-3} M) the tissue became spontaneously active with regular spikes followed by contractions.

As shown in Fig. 3*A*, application of a single field stimulus of short duration (50 μsec) produced an action potential followed by a twitch contraction in normal Krebs solution. The action potential and twitch contraction induced by field stimulation were blocked by tetrodotoxin (10^{-7} M). Application of small outward current pulses of 2 sec duration produced electrotonic potentials. If the intensity was increased, spike potentials were superimposed on the electrotonic potentials and these were followed by contraction (Fig. 3*B*). In the presence of tetrodotoxin (10^{-7} M) spike

potentials with contraction could still be evoked suggesting that these resulted from direct stimulation of the muscle cells. Thus, the action potential followed by twitch contraction observed in response to field stimulation with short duration must have arisen from stimulation of nerve fibres in the muscle tissue. The characteristics of these nerve-induced responses were studied by varying the parameters of stimulation and application of drugs.

A spike of maximum amplitude was evoked by a single stimulus of 7×10^{-6} A intensity and 50 μsec duration (Fig. 4*A*). The peak of the spike occurred within

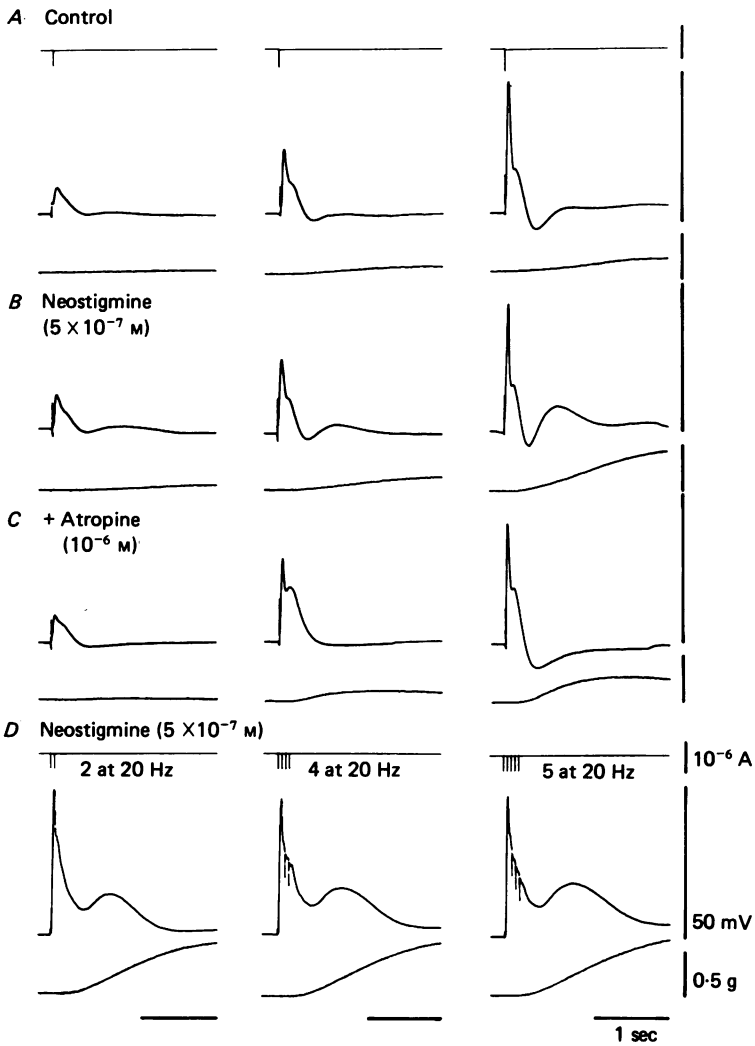

Fig. 4. Electrical and mechanical responses of the rabbit bladder to field stimulation (*A*) and the effects on these of neostigmine (*B*) and atropine (*C*). Three different intensities of stimulation (pulse width 50 μsec) were used and the responses were recorded in the double sucrose-gap. The late depolarization was enhanced by neostigmine and abolished by atropine. *D*, in a different preparation, 2, 4 and 5 pulses at 20 Hz were given in the presence of neostigmine. Repetitive stimulation increased the amplitude of the late depolarization and of the contraction.

50 msec of stimulation and the recovery could be divided into two parts with an initial rapid phase followed by a slow repolarization towards the resting membrane level. This was frequently followed by hyperpolarization and a late depolarization which had a latency of 250 msec and duration of 600–1200 msec. However, in some preparations the hyperpolarization or late depolarization was not apparent and in others the late depolarization occurred before repolarization was complete so that any hyperpolarization was obscured. With decreasing intensity of stimulation, the amplitude of the spike and late depolarization was reduced until eventually only an early depolarization without spike was seen (Fig. 4*A*). This was presumably an excitatory junction potential (e.j.p.) resulting from local action of neurotransmitter. This response lasted about 500 msec and had a threshold at a current intensity of about 2×10^{-6} A.

Fig. 4 also shows the effects of neostigmine (5×10^{-7} M) and atropine (10^{-6} M) on the nerve-induced responses. In the presence of neostigmine the amplitude of the late depolarization was markedly enhanced and, although a superimposed spike potential was never seen, the amplitude and duration of the contraction were increased. Atropine abolished this late depolarization but had no effect on the spike or early depolarization. The hyperpolarization tended to become more obvious when atropine was present. Repetitive stimulation at 20 Hz in the presence of neostigmine ($3{\cdot}3 \times 10^{-7}$ M) produced a further increase in amplitude of the late depolarization and contraction but again no spike was seen (Fig. 4*D*). These results suggest that the late depolarization is a muscarinic response resulting from stimulation of cholinergic nerves.

The properties of the early responses

In an attempt to identify the transmitter causing the initial e.j.p., a number of antagonists were applied in the presence of atropine (10^{-6} M). In ten to fifteen preparations, phentolamine ($3{\cdot}5 \times 10^{-5}$ M) and propranolol ($3{\cdot}8 \times 10^{-5}$ M) produced no change in the e.j.p., spike or contraction (Fig. 5) and guanethidine (10^{-5} M) was also without effect (Fig. 6), suggesting that noradrenaline is not involved. Methysergide (10^{-5} M), cimetidine (10^{-5} M) and mepyramine (10^{-5} M) also failed to reduce the responses so that it is unlikely that 5-hydroxytryptamine or histamine is the transmitter.

The effects of quinidine and theophylline, which are believed to selectively block P_2 and P_1 purinergic receptors (Burnstock, 1978), were observed (Fig. 5). Quinidine (5×10^{-5} M) had no effect on the electrical events but slightly reduced the amplitude of contraction ($n = 7$). Theophylline (5×10^{-5} M), on the other hand, often slightly increased contraction without altering the electrical response ($n = 9$). This therefore suggests that they may have direct actions on the contractile properties of the smooth muscle.

Repetitive stimulation at frequencies greater than 20 Hz produced summation of submaximal responses so that a spike could be initiated on the e.j.p. or its amplitude increased. With stimulation at frequencies of between 2 Hz and 0·5 Hz in the presence of atropine ($3{\cdot}5 \times 10^{-6}$ M) and guanethidine (1×10^{-5} M) each stimulus produced an e.j.p. with depression of successive responses. Although the intensity could be adjusted so that no spike could be seen, the amplitude of the response was small. In

order to record a larger e.j.p. without spike, the spike was, therefore, differentially blocked with the Ca^{2+} antagonist nicardipine (Fig. 6). At 2×10^{-6} M nicardipine, the spike amplitude was considerably reduced but the e.j.p., as indicated by the inflexion on the falling phase, was only slightly smaller. Further reduction in the spike and e.j.p. occurred at a dose of 5×10^{-6} M. By decreasing the intensity of stimulation, a large e.j.p. could still be obtained with relatively little or no spike. Under these circumstances the rate of depression was the same as in the control. There was no

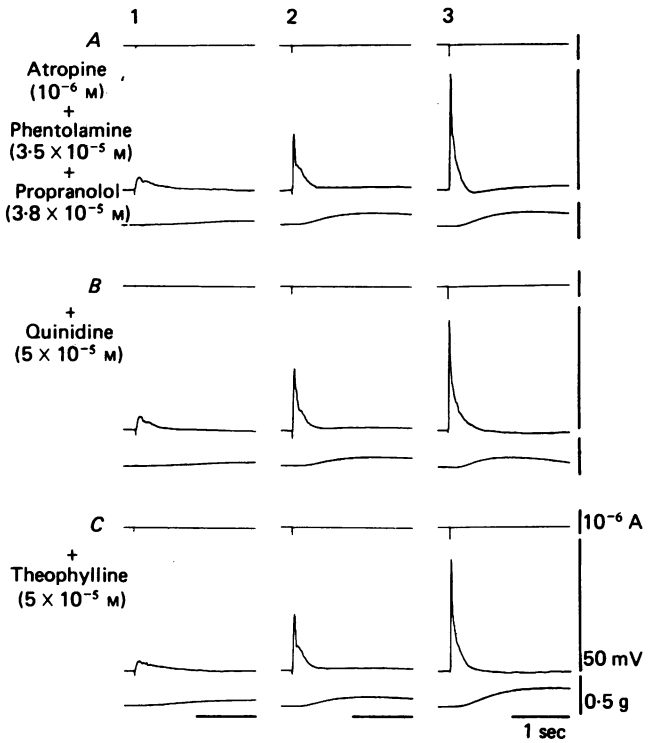

Fig. 5. The effects of quinidine and theophylline on responses to field stimulation of rabbit bladder recorded in the double sucrose-gap. *A*, field stimulation (top trace) with three intensities (pulse width 50 μsec) in the presence of atropine, phentolamine and propranolol produced e.j.p., with superimposed spike at higher intensities. *B* and *C*, addition of neither quinidine nor theophylline reduced the amplitude of the electrical responses.

significant difference between amplitudes of e.j.p. before and after addition of nicardipine (5×10^{-6} M), with the mean amplitudes after 20 sec falling to 75 and 78 % of the original at 0·5 Hz ($n = 7$, $P > 0{\cdot}1$) and to 23 and 26 % at 2 Hz ($n = 8$, $P > 0{\cdot}2$).

The hyperpolarization, which could only sometimes be seen in control Krebs solution (Figs. 3, 4), was often revealed when the late depolarization was abolished by atropine (Figs. 5, 6, 7). It remained in the presence of guanethidine (10^{-5} M) (Fig. 6). Although in a few preparations, some reduction was seen after application of high concentrations of propranolol ($3{\cdot}8 \times 10^{-5}$ M), this was not consistent. The amplitude of the hyperpolarization was reduced as the intensity of stimulation was decreased but there was also some reduction on application of nicardipine (5×10^{-6} M) when the

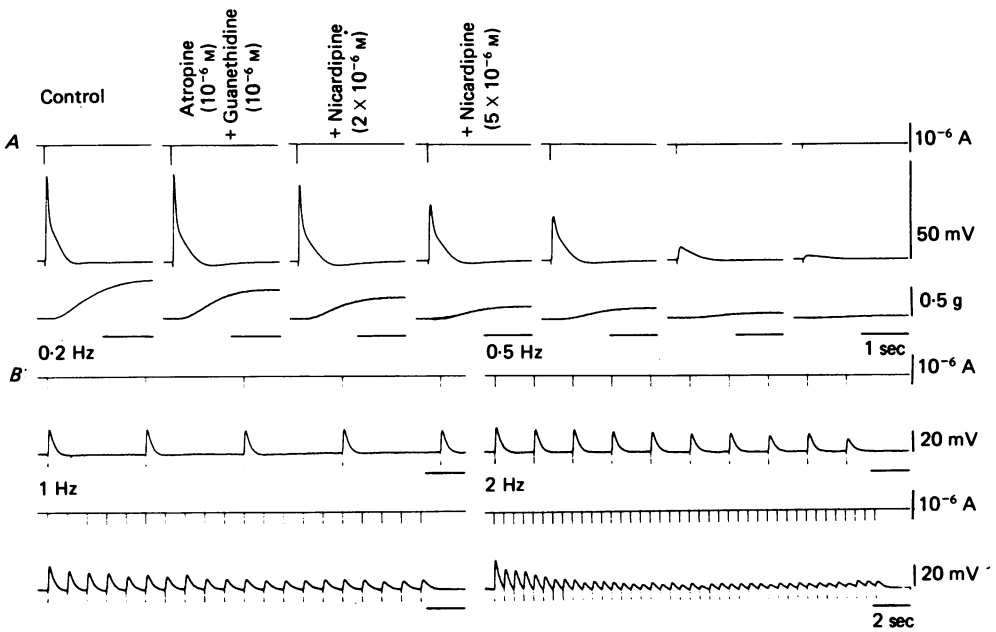

Fig. 6. *A*, the effect of the Ca^{2+} antagonist, nicardipine, on the responses to field stimulation. In the first four records the stimulus intensity was constant. Nicardipine reduced the amplitude of the spike with little change in the e.j.p. The intensity was then decreased in three steps so that only the e.j.p. remained. *B*, depression of successive e.j.p. produced by repetitive stimulation at 0·2, 0·5, 1 and 2 Hz in the presence of nicardipine (5×10^{-6} M). The intensity of stimulation was adjusted so that only the e.j.p. occurred.

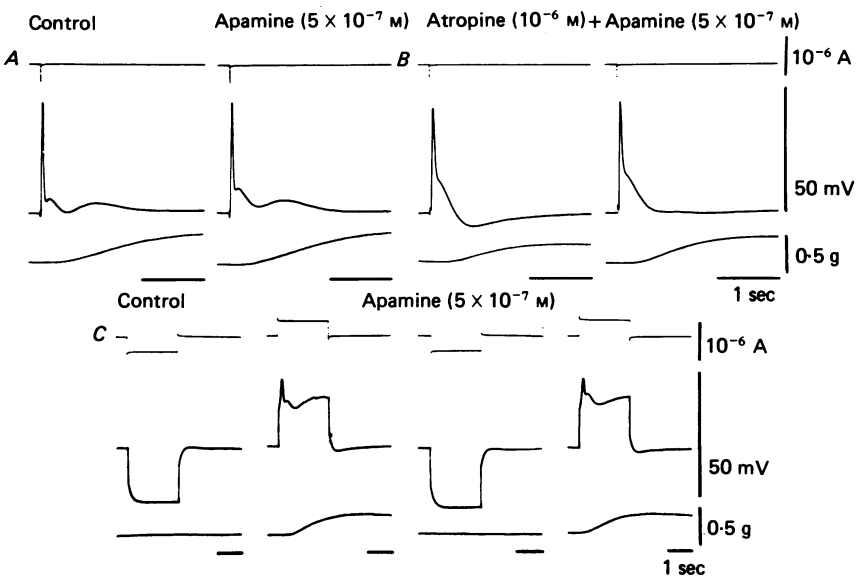

Fig. 7. The effect of apamine on the responses to field stimulation and direct muscle stimulation of rabbit bladder strips. *A*, apamine increased the amplitude of the e.j.p. *B*, in another preparation, the hyperpolarization revealed by atropine was abolished by apamine. *C*, the amplitude of the electrotonic potential produced by hyperpolarizing current of 2 sec duration was slightly increased, and the amplitude of the spike produced by depolarizing current was increased.

intensity of stimulation was kept constant (Fig. 6). This therefore suggests that it is at least partly associated with the spike. Until the initial e.j.p. is selectively inhibited, it will not be possible to determine whether direct action of a transmitter is also involved.

Fig. 7 shows the effect on the responses of apamine, a polypeptide which has been shown to block the inhibitory junction potential (i.j.p.) in the taenia coli of guinea-pig (Vladimirova & Shuba, 1978). Apamine (5×10^{-7} M) produced an increase in the amplitude of the e.j.p. (Fig. 7*A*) and abolished the hyperpolarization recorded in the presence of atropine (Fig. 7*B*). Similar effects were produced by TEA (2×10^{-3} M) which inhibits K^+ conductance changes. By measuring electrotonic potentials

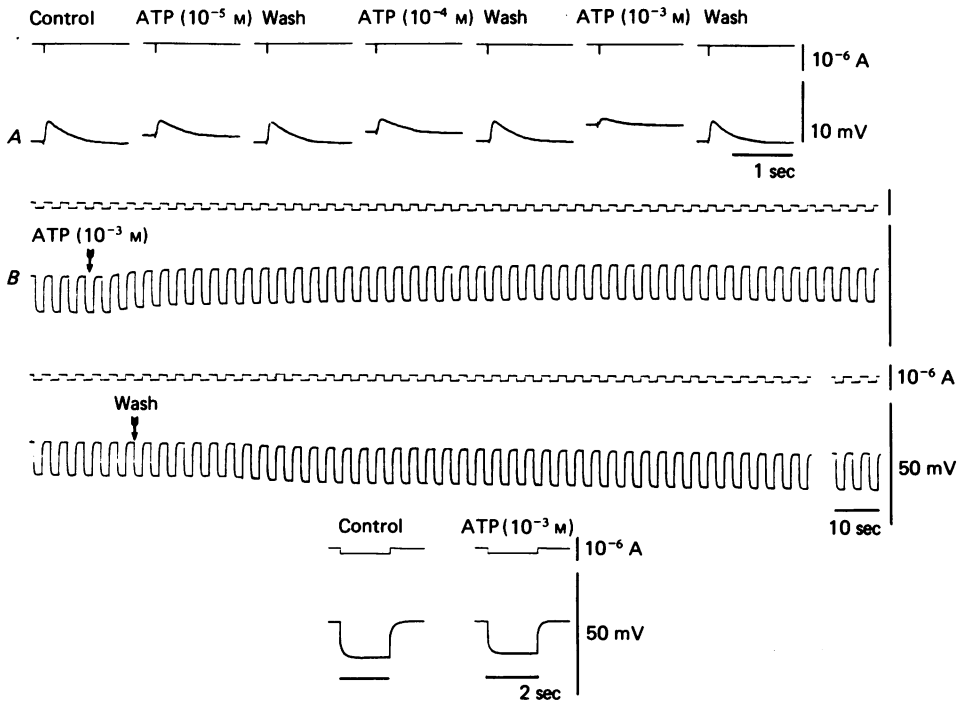

Fig. 8. The effect of ATP on the e.j.p. and membrane resistance recorded from the rabbit bladder with the double sucrose-gap. *A*, application of ATP reversibly reduced the amplitude of the e.j.p. recorded in the presence of nicardipine (5×10^{-6} M). *B*, small hyperpolarizing current pulses of 2 sec duration and constant intensity were applied. In the presence of ATP the amplitude of the resulting electrotonic potentials were reduced, indicating a decrease in membrane resistance, and the membrane was depolarized.

produced by small hyperpolarizing current pulses of 2 sec duration, it was found that apamine (5×10^{-7} M) slightly increased membrane resistance and the amplitude of spike evoked by direct stimulation of the muscle with depolarizing pulses (Fig. 7*C*). TEA (2×10^{-3} M) produced a larger increase in amplitude of the spike.

Effects of ATP on membrane properties

In order to test whether the e.j.p. was reduced after desensitization of the tissue to ATP, single stimuli of small intensity were applied in the presence of nicardipine (5×10^{-6} M) (Fig. 8*A*). ATP was added to the perfusing fluid and, 15 min later,

stimulation was repeated. The ATP was then withdrawn. ATP itself produced depolarization and, compared with control values, the amplitude of the e.j.p. was reduced dose dependently. At ATP concentrations of 10^{-5}, 10^{-4} and 10^{-3} M the mean amplitudes of e.j.p. were 79·4±7·9%, 63·5±3·4% and 40·0±13·4% (mean±S.D. $n = 5$–6) of control values respectively. The effect was completely reversible with recovery to the original amplitude occurring within 15–20 min after removing ATP from the perfusing fluid.

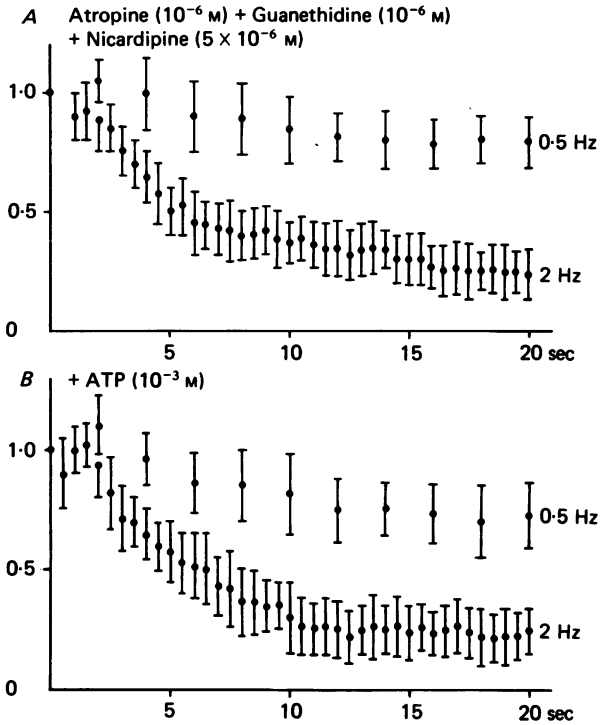

Fig. 9. The rates of depression recorded from three to five trains of stimuli at 0·5 and 2 Hz. Each point is the mean and the bars are 2 × S.D. The first response was taken as relative amplitude of 1. The rate of depression was identical before (*A*) and after (*B*) application of ATP. The experiment was carried out in the presence of atropine, guanethidine and nicardipine ($n = 7$–10).

The rate of depression was similar in the control and in the presence of ATP. In Fig. 9 the mean relative amplitude of successive e.j.p. at 0·5 and 2 Hz is shown with the initial response expressed as 1·0. There was no significant difference in relative amplitudes after 20 sec (79 and 73% of original at 0·5 Hz, and 23·5 and 25% at 2 Hz; $n = 7$–10, $P > 0·1$, $> 0·1$).

As well as decreasing the amplitude of the e.j.p., ATP depolarized the membrane. It is therefore possible that the reduction in amplitude of the e.j.p. resulted from changes in membrane properties rather than from desensitization of specific receptors. These properties were therefore studied in greater detail. The membrane resistance (r) was measured by application of small hyperpolarizing current pulses of 2 sec duration (Fig. 8*B*). The amplitude of the resulting electrotonic potential (e) was reduced from 14·5 mV in the control to 13 mV in the presence of 10^{-3} M-ATP. This

represents a fall in resistance to 80 % of the control since $(e_1/e_2)^2 = r_1/r_2$. Corresponding reductions of 87 and 93 % were produced by ATP at concentrations of 10^{-4} and 10^{-5} M, respectively ($n = 7$–10).

Although membrane potentials cannot be measured accurately by the double sucrose-gap method, a depolarization of several millivolts was apparent on addition

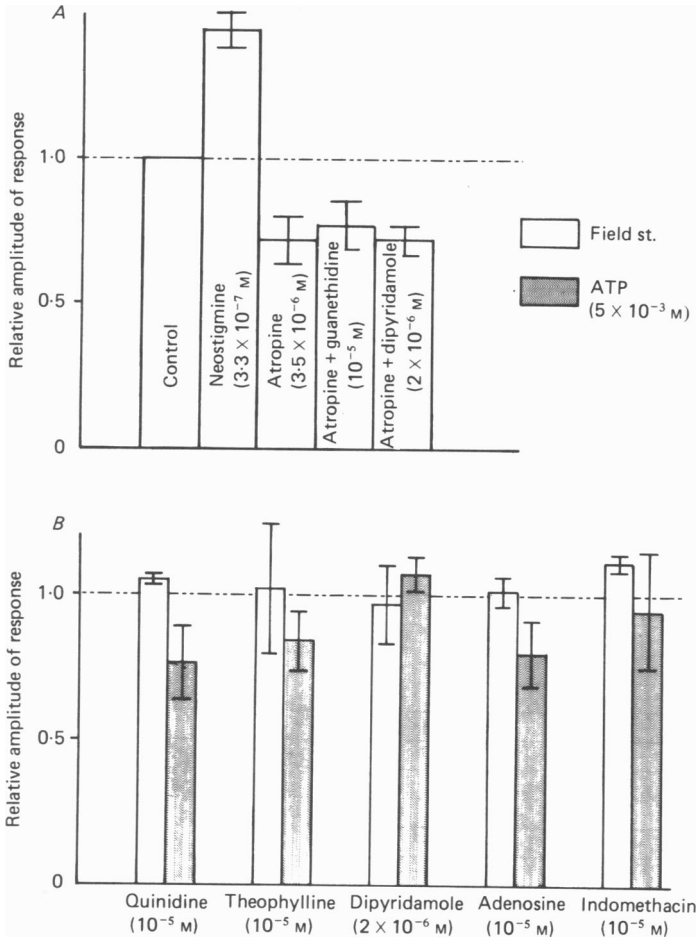

Fig. 10. Histograms to show the effects of various drugs on the amplitude of contractions of rabbit bladder strips produced in response to field stimulation (nine stimuli at 20 Hz) or ATP. *A*, the contraction to field stimulation was enhanced by neostigmine and reduced by atropine. Neither guanethidine nor dipyridamole produced further change. *B*, compared with control values in Krebs solution (relative amplitude = 1·0) responses to field stimulation were unchanged but quinidine, theophylline and adenosine all reduced responses to ATP applied directly to the bath. Bars indicate 2 × S.D., measured in six to seven preparations.

of 10^{-3} M-ATP (Fig. 8*B*). This was confirmed by the use of micro-electrodes. In the control the mean resting membrane potential was −37·6–4·4 mV ($n = 15$). In many cells addition of 10^{-3} M-ATP produced a transient increase in spike frequency followed by depolarization of up to 5 mV when the spike frequency was similar to or less than control values.

It has been reported that adenosine, a metabolite of ATP, has an inhibitory action on the urinary bladder. Dipyridamole, which prevents uptake of adenosine, either fails to affect contractions (Dean & Downie, 1978) or reduces responses, presumably by potentiating adenosine action (Burnstock, Cocks, Crowe & Kasakov, 1978). The effects of these two agents on the e.j.p. were therefore tested in the presence of atropine and guanethidine. At a concentration of 2×10^{-6} M, dipyridamole produced no change in the amplitude of the e.j.p. or spike (mean amplitude of e.j.p. = $95{\cdot}8 \pm 12{\cdot}8\%$ of control $n = 8$–13) and the contraction was unchanged. Adenosine at 10^{-4} M also had no effect.

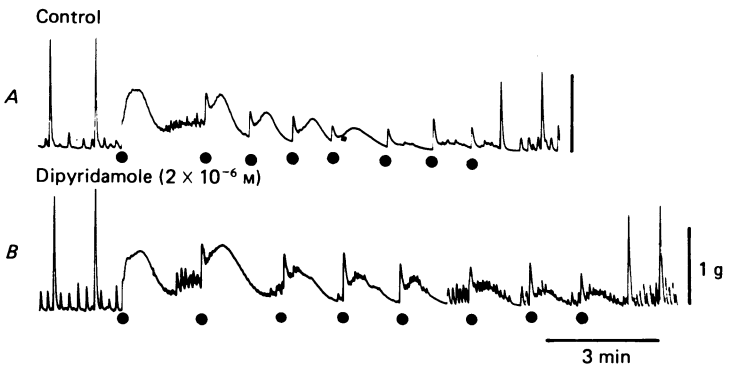

Fig. 11. Desensitization of rabbit bladder to ATP and the effect on this of dipyridamole. *A*, after obtaining two mechanical responses to field stimulation (nine stimuli at 20 Hz), ATP (10^{-3}M) was added to the bath. When its effects had worn off due to dilution with perfusing fluid, more ATP was applied. The response to ATP could be divided into phasic and tonic components, the second of which decreased in amplitude with each application. There was also some reduction in the amplitude of the subsequent response to field stimulation. *B*, dipyridamole increased spontaneous activity but the effect of ATP was unchanged. Each application of ATP is marked by a dot.

Mechanical responses

In order to confirm the contractile responses recorded in the double sucrose-gap, where activity of a length of tissue less than 0·5 mm can be observed, mechanical responses to field stimulation and to ATP were investigated in an organ bath. In addition, the effects of desensitization by repeated application of ATP were recorded. Supramaximal field stimulation was carried out with nine stimuli at 20 Hz with a pulse duration of 300 μsec. Compared with control responses in Krebs solution, neostigmine ($3{\cdot}3 \times 10^{-7}$ M) increased the amplitude of contraction to 144% ($n = 6$) and atropine ($3{\cdot}5 \times 10^{-6}$ M) reduced them to 72% ($n = 6$) (Fig. 10*A*). Guanethidine (10^{-5} M) or dipyridamole (2×10^{-6} M) produced no further reduction ($n = 7$).

Field stimulation at 20 Hz produced a large contraction which decayed rapidly to the resting level at the end of the train (Fig. 11). Application of ATP (10^{-3} M) directly to the bath produced contraction which could be divided into phasic and tonic components (Fig. 11). Under the present conditions of stimulation the mean amplitude of the tonic component was $61{\cdot}2 \pm 20{\cdot}5\%$ ($n = 26$) of the response to field stimulation. Fig. 10*B* shows the effect of some drugs on the amplitude of these contractions. In no case were the responses to field stimulation significantly reduced but theophylline, quinidine and adenosine all reduced responses to ATP.

With repetitive application of ATP, a decrease in successive responses was obtained, especially of the second component (Fig. 11*A*). There was also a significant but much smaller reduction in the response to field stimulation (82·3 ± 12·7 %, $n = 26$ for field stimulation; 65·7 ± 19·1 %, $n = 6$ for the first component and 20·8 ± 11·1 %, $n = 26$ for the second). In the presence of quinidine and theophylline, ATP produced a larger reduction in the response to field stimulation but in the presence of dipyridamole no reduction occurred.

DISCUSSION

In the present experiments, two distinct depolarizing responses to nerve stimulation were obtained by the double sucrose-gap method. These were an initial e.j.p., on which a spike was superimposed, and a late depolarization. Only the second of these was enhanced by neostigmine and abolished by atropine suggesting that the bladder is innervated by both cholinergic and non-cholinergic excitatory fibres.

The latency of the late depolarization was 250 msec. This compares with latencies for muscarinic responses to nerve stimulation of other smooth muscles, which are usually 100 msec or more (Gillespie, 1962; Ito & Kuriyama, 1971). Similar latencies occur in response to ionophoretically applied acetylcholine, suggesting that they are due to events in the smooth muscle cells (Purves, 1974). Micro-electrode recording indicated that regular spontaneous action potentials occur in the smooth muscle cells and acetylcholine produced slight depolarization and increase in frequency. The effect of the late nerve-induced depolarization, which lasted for up to 1 sec, would be to increase contraction by modulating this spontaneous activity.

The initial e.j.p. showed marked depression with repetitive stimulation at frequencies of 0·2 to 2 Hz. On the other hand, a single stimulus was adequate to produce an action potential. It is therefore possible that the non-cholinergic nerves produce large contractions of rapid onset but sustained contraction is due to the more prolonged action of acetylcholine released from cholinergic nerves.

Except with low intensities of stimulation, there was a spike superimposed on the e.j.p. and this was usually followed by hyperpolarization. The shape of the spontaneous action potentials recorded with micro-electrodes, and also of the conducted action potentials resulting from direct muscle stimulation, showed some variation. Frequently, however, the spike was followed by a slow depolarization lasting up to 200 msec and sometimes by a long after-hyperpolarization. Ursillo (1961) also reported that spikes in the rabbit detrusor were associated with waves of depolarization and marked hyperpolarization. The recordings of compound action potentials in the double sucrose-gap must include these components. In order to study the pure e.j.p., therefore, either stimulation at low intensity was used or recordings were made in the presence of nicardipine. In basilar artery this agent inhibited the Ca^{2+} channel producing the spike in the smooth muscle cells at a concentration of 3×10^{-7} M but had little effect on the release of transmitter from the nerves (Fujiwara & Kuriyama, 1983). In guinea-pig bladder, the action potentials are due to Ca^{2+} entry (Creed, 1971), and the present experiments suggest that in the rabbit bladder the smooth muscle action potentials were selectively blocked by nicardipine.

The present experiments indicated that the e.j.p. was not due to release of

noradrenaline, 5-hydroxytryptamine or histamine. ATP produced rapid phasic contraction followed by a larger tonic contraction. The latter, in particular, was suppressed by repetitive application of ATP. The nerve-induced response was only slightly reduced after a series of ATP contractions confirming results reported by Ambache & Zar (1970). It should be noted, however, that Burnstock, Dumsday & Smythe (1972) reported that complete tachyphylaxis to ATP was only produced when ATP was left in contact with the tissue. Under these circumstances the nerve-induced mechanical response was also depressed (Dean & Downie, 1978). In the present experiments prolonged application of ATP produced depolarization of the membrane and a decrease in membrane resistance. This itself could explain the reduction in amplitude of the e.j.p. if this is due to a change in permeability of the membrane produced by transmitter action.

Dipyridamole is believed to act by preventing uptake of adenosine, a metabolite of ATP. It would therefore be expected to either potentiate the action of ATP or unmask a response to adenosine. However, the contractions to ATP or to nerve stimulation are unaffected by dipyridamole (Ambache & Zar, 1970; Dean & Downie, 1978). This could be because the excitatory response to ATP and inhibitory response to adenosine are both equally potentiated (Burnstock, Cocks Crowe & Kasakov, 1978), but it is unlikely that such opposing actions would not be seen when recording membrane potential changes to field stimulation, if the excitatory transmitter is ATP.

From the present experiments it is not possible to conclude whether the hyperpolarization is due to transmitter action or entirely to after-hyperpolarization of the spike. The reduction by apamine, which is known to block the i.j.p. in other tissues (Vladimirova & Shuba, 1978; Shuba & Vladimirova, 1980), can be explained by a non-specific decrease in K^+ conductance of the smooth muscle cells. A similar action on nerve terminals could prolong transmitter release and hence increase the amplitude of the e.j.p.

In the alimentary canal, recent investigations have revealed the presence of non-adrenergic, non-cholinergic excitatory innervation to many parts including longitudinal (Bauer & Kuriyama, 1982) and circular (Bywater, Holman & Taylor, 1981) muscle layers of guinea-pig ileum. Furthermore, tracheobronchial smooth muscle of guinea-pig and cat contains non-adrenergic, non-cholinergic inhibitory nerve fibres (Ito & Takeda, 1982). Thus a wide distribution of such excitatory or inhibitory nerve fibres to visceral smooth muscle tissue is evident. The mechanisms involved in the non-adrenergic, non-cholinergic neuro-effector transmission and the identification of transmitter substances in the visceral smooth muscles must be clarified in future investigations.

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