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Electrical properties of smooth muscle in the guinea-pig urinary bladder

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1. The effects of transmural nerve stimulation were examined on preparations of detrusor smooth muscle from guinea-pig urinary bladder using intracellular recording techniques. Most recordings were made from preparations in which spontaneous and evoked action potentials had been inhibited by nifedipine (10 μM), a dihydropyridine that blocks L-type Ca^{2+} channels.

2. Supramaximal stimuli evoked excitatory junction potentials (EJPs) which could be divided into three basic types. Type 1 EJPs had short latencies (< 30 ms) and fast rise times (< 60 ms). Type 2 EJPs consisted of two components: a small depolarization that was followed by a second depolarization with a faster rise time. In a third type of cell, at high strengths of stimulation, EJPs resembled type 1 EJPs but at lower strengths of stimulation were similar in time course to type 2 EJPs.

3. All EJPs were abolished by tetrodotoxin (1 μM) and reduced by ω-conotoxin (0·1 μM), but were unaffected by hexamethonium (0·1 mM), suggesting that they result from the release of transmitter from post-ganglionic nerve fibres. All responses persisted in the presence of atropine (1 μM) but were abolished following the desensitization of P_2-purinoceptors with α,β-methylene ATP (m-ATP; 10 μM).

4. Spontaneous excitatory junction potentials (SEJPs) were also recorded from most cells. SEJPs were similar in appearance to fast single-component EJPs; however, in general they had a briefer time course. SEJPs persisted in the presence of tetrodotoxin (1 μM).

5. The electrical properties of urinary bladder smooth muscle were also examined. Voltage changes induced by point current injection into cells had fast rates of rise and decay (time constant, 5–20 ms). The input resistance of cells ranged between 12 and 108 MΩ. When recordings were taken from cells near the point of current injection, resultant electrotonic potentials could be detected in only a small proportion of cells.

6. The results are discussed in relation to the idea that transmural nerve stimulation in the guinea-pig urinary bladder causes the activation of at least two different membrane conductances. Cells appear to be electrically coupled with one another. However, it is likely that coupling exists within discrete bundles of the smooth muscle.

The mammalian urinary bladder receives a dense excitatory innervation originating from the sacral parasympathetic nucleus (Langley & Anderson, 1895). In most mammals, low frequency stimulation of the intrinsic nerves innervating bladder smooth muscle evokes a membrane depolarization, or excitatory junction potential (EJP), on which is superimposed an action potential spike (Creed, Ishikawa & Ito, 1983; Fujii, 1988; Brading & Mostwin, 1989; Creed, Callahan & Ito, 1994). In the guinea-pig, these membrane potential changes are thought to result primarily from the purinergic activation of a non-selective cation conductance and the subsequent opening of voltage-dependent L-type Ca^{2+} (Ca_L) channels (Klöckner & Isenberg, 1985; Inoue & Brading, 1990; Creed *et al.* 1994). The resultant influx of Ca^{2+} brings about contraction of the detrusor smooth muscle.

Most studies examining the membrane potential changes underlying nerve-evoked contractions of detrusor smooth muscle have done so using the double sucrose-gap technique (Creed *et al.* 1983; Hoyle & Burnstock, 1985; Fujii, 1988; Creed, Ito & Katsuyama, 1991). This technique only allows

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investigation of the membrane potential changes occurring within the total syncytium and therefore provides no information on the variability of responses between cells and is unable to detect spontaneous changes in membrane potential which may occur within individual cells. Although cells of the guinea-pig urinary bladder are electrically coupled together to form a syncytium (Creed, 1971), the impedance of this tissue is high (Brading, Parekh & Tomita, 1989), suggesting that cells may not be as extensively coupled as other smooth muscle tissues. The present study used intracellular recording techniques to examine further the membrane potential changes evoked by transmural nerve stimulation of guinea-pig detrusor smooth muscle. The electrical properties of the smooth muscle were also examined by measuring membrane potential changes induced by point intracellular current injection.

METHODS

Male guinea-pigs, weighing 200–250 g, were killed by cervical dislocation and exsanguination. Longitudinal strips of detrusor smooth muscle, measuring approximately 2 by 10 mm, were taken from the dorsal surface of the bladder starting at the level of the two ureteric openings and reaching up to the bladder dome. After removal of the mucosa, muscle strips were pinned out in a shallow recording chamber (bath volume, 2 ml) using pins cut from 100 μm tungsten wire. The base of the recording chamber was coated with Sylgard silicone resin (Dow Corning Corporation, Midland, MI, USA). The urethral end of the preparation was placed so that it lay between two platinum electrodes (wire diameter, 1 mm). Intrinsic nerves were stimulated using voltages of 1–90 V and 0·01–1·0 ms pulse widths.

Intracellular recordings were made using conventional techniques with fine glass microelectrodes (resistance, 100–210 MΩ) filled with 0·5 M KCl. All membrane potential records were low-pass filtered (cut-off frequency, 1 kHz), digitized and stored on disk for later analysis. The passive electrical properties of cells were determined using a single electrode voltage clamp (Axon Instruments); following neutralization of the tip capacitance, both hyperpolarizing and depolarizing current was passed through the recording electrode (500 ms duration). In experiments in which the degree of electrical coupling between cells was examined, the preparation was impaled with two independent microelectrodes and the distance and orientation of the two electrodes were determined using an inverted compound microscope (see Bywater, Campbell, Edwards & Hirst, 1990). Preparations were continuously perfused with a physiological saline (composition, mM: NaCl, 120·2; KCl, 5·9; $NaHCO_3$, 15·5; NaH_2PO_4, 1·2; $CaCl_2$, 2·5; $MgCl_2$, 1·2; glucose, 11·5; gassed with 97% O_2–3% CO_2) at a rate of 3 ml min^{-1}. In most experiments, spontaneous and nerve-evoked action potentials were abolished by addition of the dihydropyridine calcium antagonist, nifedipine (10 μM), to the physiological saline. Other drugs were added to the preparation by changing the inflow line from the control solution to one containing the appropriate concentration of drug. All experiments were performed at 25 °C. At this temperature spontaneous and nerve-evoked contractions of the smooth muscle were reduced, allowing impalements to be maintained for longer periods of time.

Drugs used in this study were nifedipine hydrochloride, nicardipine hydrochloride, hexamethonium bromide, atropine sulphate, α,β-methylene adenosine triphosphate (m-ATP), tetrodotoxin, ω-conotoxin GVIA (all from Sigma Chemical Co.). In the text all drugs are expressed in concentrations of their salts.

All results are given as means ± S.E.M. unless otherwise stated. The latency of EJPs was measured from the stimulus artifact to 10% of the peak amplitude. Rise times were measured from 10 to 90% of the peak amplitude.

RESULTS

General observations

Membrane potential recordings were made from spontaneously active preparations of guinea-pig detrusor smooth muscle. Stable resting membrane potentials which ranged between −41 and −47 mV (mean, −45 ± 1 mV; $n = 11$ from 9 preparations) were, in most preparations, interrupted by the spontaneous generation of action potentials (Fig. 1*A* and *C*). Spontaneous action potentials were always associated with contraction of the tissue. The amplitude of action potentials ranged between 19 and 40 mV (mean, 26·1 ± 2·8 mV; $n = 7$). Action potentials were preceded by a membrane depolarization of 5–10 mV, which lasted approximately 10–20 ms, and were followed by after-hyperpolarizations of some 3–10 mV in amplitude. The frequency of spontaneous action potential generation varied between 1 and 10 action potentials min^{-1}.

Membrane potential changes evoked by intrinsic nerve stimulation

In all cells recorded from, application of a brief supra-maximal impulse (30 V, 0·5 ms), to activate intrinsic nerves of the muscle selectively, evoked an action potential which was associated with contraction of the smooth muscle. Evoked responses were similar in time course to spontaneous action potentials (Fig. 1*A* and *B*). Action potentials had amplitudes of 51·0 ± 1·9 mV ($n = 34$ from 9 preparations). Evoked responses were preceded by a membrane depolarization and, like spontaneous action potentials, were always followed by an after-hyper-polarization of between 2 and 15 mV (mean, 7·8 ± 0·6 mV; $n = 33$ from 9 preparations). Such after-hyperpolarizations are abolished by apamin and have therefore been attributed to the activation of Ca^{2+}-dependent K^+ channels (Creed *et al.* 1983; Fujii, Foster, Brading & Parekh, 1990). As the stimulus strength was decreased, the amplitude of nerve-evoked action potentials was depressed and their rise time slowed. In a small number of cells, subthreshold depolarizations were evoked which failed to generate the active component of the action potential. Even though such strengths of stimulation evoked EJPs in these cells, action potentials were presumably activated in other parts of the tissue, as the resultant contractions often caused displacement of the recording electrode. Therefore, due to the difficulty in recording from these cells, a detailed

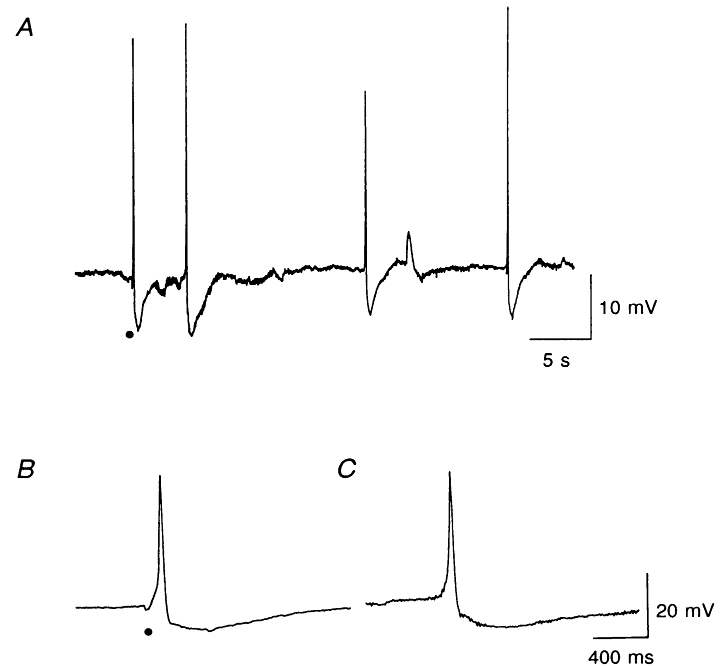

Figure 1. Spontaneous and evoked action potentials recorded from detrusor smooth muscle of the guinea-pig urinary bladder

A, an action potential evoked by a brief single supramaximal stimulus (30 V, 0·5 ms; ●), followed by spontaneous action potentials. Also note the presence of a small spontaneous membrane depolarization which fails to trigger an action potential. An evoked and spontaneous action potential, recorded from the same cell, are shown in *B* and *C*, respectively, on an expanded time scale. Note that both the nerve-evoked and spontaneous action potentials have a similar time course. Both action potentials arise after an initial membrane depolarization and are followed by an after-hyperpolarization. The resting membrane potential was −44 mV. The top calibration bar refers to *A* and the lower calibration bars refer to *B* and *C*.

analysis of nerve-evoked action potentials was not undertaken.

To examine more closely the responses to nerve stimulation which led to the generation of action potentials, the dihydropyridine Ca^{2+} antagonist, nifedipine (10 μM), was added to the physiological saline. Following the addition of nifedipine, cells had resting membrane potentials of between −34 and −58 mV (mean, -46 ± 1 mV; $n = 103$). Figure 2 shows responses to a single transmural stimulus before (*A*) and after (*B*) the addition of nifedipine (10 μM) to the physiological saline; nifedipine abolished the action potential, leaving an underlying EJP. This result is similar to that obtained in the rabbit urinary bladder using the double sucrose-gap technique (Creed *et al.* 1983). However, nifedipine-resistant EJPs such as this were not recorded from all cells. Figure 2*C* shows a recording, taken from a cell in the same preparation as Fig. 2*A* and *B*, also in the presence of nifedipine. In this cell, the EJP had a larger amplitude and had two components to its rising phase. From initial observations such as these, it was apparent that there was variation in both the size and time course of responses recorded from different cells of any given preparation. To characterize such responses further the remaining experiments were performed in the presence of nifedipine (10 μM).

Properties of excitatory junction potentials

EJPs recorded from any given preparation could be fitted into three basic types. (1) Simple EJPs which had a short latency and brief time course. (2) EJPs which were biphasic in nature. (3) EJPs which at high stimulus strengths resembled type 1 EJPs but at lower strengths of stimulation became biphasic (type 2). Characteristics of the three EJP types are shown in Table 1. In most preparations, all three types of EJP were observed.

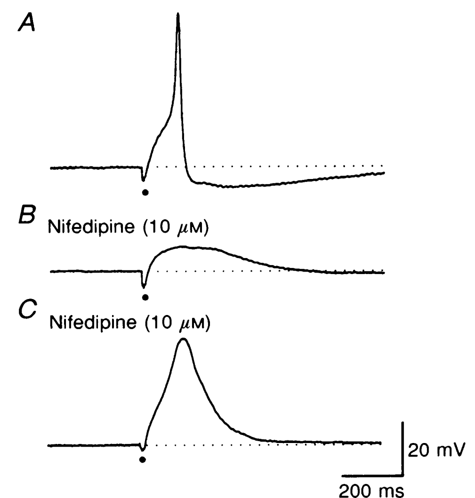

Figure 2. Effect of nifedipine on nerve-evoked action potentials recorded from detrusor smooth muscle of guinea-pig urinary bladder

The upper two traces show responses evoked by a single supramaximal stimulus (30 V, 0·5 ms) before (*A*) and after (*B*) the addition of nifedipine (10 μM) to the physiological saline. After the addition of nifedipine the action potential is abolished, leaving an underlying membrane depolarization or EJP which has a simple time course. *C*, a response recorded from a second cell, also in the presence of nifedipine, at a similar distance from the stimulating electrodes. In this cell, the underlying EJP is larger in amplitude and has two components to its rising phase. The resting membrane potential of both cells was −42 mV. The calibration bars refer to all traces.

Brief, short latency EJPs were recorded from approximately 30% of cells (Fig. 3*A*). Stimulation with a single supramaximal impulse evoked EJPs which had amplitudes of between 4·8 and 53·1 mV and latencies of between 15 and 46 ms ($n = 48$ from 38 preparations). The time course of decay of EJPs could be described by a single exponential with a time constant ranging between 49 and 196 ms. In these cells, reducing either the pulse width, or strength of stimulation, decreased the amplitude of EJPs with little change to either their latency or rise time (Fig. 3*A*).

Biphasic depolarizations (type 2) were recorded from a further 30% of cells (Fig. 3*B*). EJPs of this type consisted of an initial small membrane depolarization which appeared to initiate a second depolarization with a faster rise time. Single supramaximal stimuli evoked responses with total amplitudes ranging between 12·8 and 47·8 mV and latencies of between 17 and 126 ms ($n = 48$ from 34 preparations; Table 1). In these cells, as the stimulus strength was decreased there was a prolongation of the time course of the response; the initial depolarization had a slower rise time and the onset of the second depolarization was delayed. Both components of such EJPs persisted in the presence of either higher concentrations of nifedipine (20 μM), or after the addition of nicardipine (10 μM; $n = 3$). It is therefore unlikely that the second, apparently voltage-dependent, component of the response is due to the inability of nifedipine to block all Ca_L channels.

In the remaining cells EJPs were evoked which, at high strengths of stimulation (10–30 V, 0·5 ms), resembled type 1 EJPs (Table 1) but at lower stimulus strengths (< 10 V, 0·5 ms) were similar to type 2 EJPs (Fig. 3*C*); an initial depolarization preceded a further depolarization with a faster rising phase. At supramaximal strengths of stimulation, such EJPs had amplitudes ranging between 4·8 and 55·9 mV and latencies of between 15 and 42 ms ($n = 63$ from 42 preparations). Unlike single-component (type 1) EJPs, these responses could be graded gradually by decreasing either the stimulus strength or pulse width. As the stimulus strength was reduced, the two components of the EJP became more evident and the initiation of the second depolarization was delayed. Eventually the stimulus strength was such that only the initial component of the response could be evoked. Similar results were obtained if the stimulation voltage was kept constant (30 V), but the stimulus pulse width was reduced (< 0·1 ms).

Neither single- (type 1) nor two-component (type 2) EJPs were affected by the ganglion-blocking drug, hexamethonium (0·1 mM; $n = 4$), suggesting that both responses result from the stimulation of post-ganglionic nerve fibres. However, all responses (stimulus parameters: 1–90 V, 0·01–1·0 ms) were abolished after addition of tetrodotoxin (1 μM; Fig. 4). Responses were reduced, but never abolished, by the addition of ω-conotoxin GVIA (0·2 μM), a blocker of N-type Ca^{2+} (Ca_N) channels located in nerve terminals. In control solution the amplitudes of single-component EJPs were 38·2 and 26·5 mV; after perfusion of ω-conotoxin their amplitudes were 26·5 and 8·1 mV, respectively. Two-component EJPs had amplitudes of 36·6 ± 7·0 and 17·4 ± 11·1 mV ($n = 4$) before and after the addition of ω-conotoxin, respectively. It is unclear why ω-conotoxin failed to abolish either type of EJP. However,

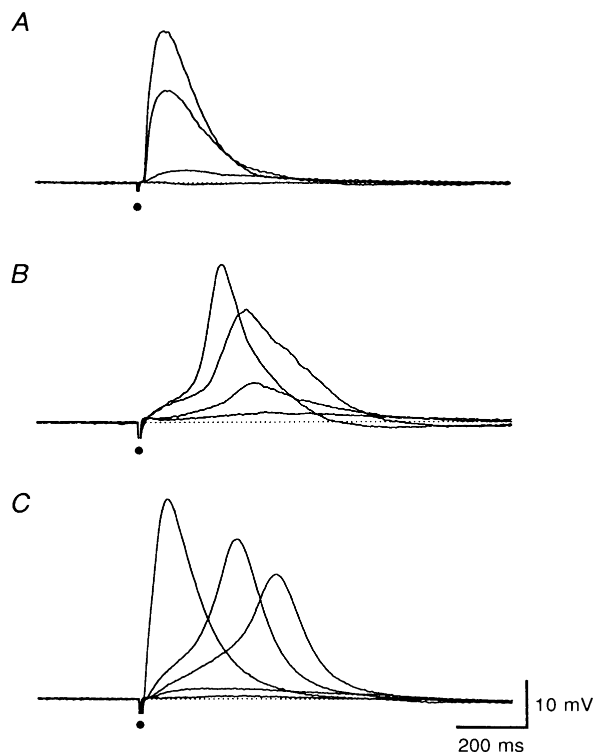

Figure 3. Comparison of the different classes of EJP recorded from detrusor smooth muscle of guinea-pig urinary bladder

Each series of traces shows responses to a single brief stimulus and the effect of decreasing the strength of stimulation (2–40 V, 0·5 ms) on the amplitude and time course of the resultant EJP. *A*, an EJP (type 1) in which lowering the strength of stimulation resulted in a decrease in the amplitude, with little change to the time course, of the evoked responses. *B*, an EJP (type 2) which consisted of two components; an initial small membrane depolarization appears to trigger a second depolarization. *C*, an EJP (type 3) which, at supramaximal strengths of stimulation, resembled a type 1 EJP but at lower strengths of stimulation consisted of two components, similar to a type 2 EJP. In all records nifedipine (10 μM) was present throughout. The resting membrane potential in *A*, *B* and *C* was −52, −45 and −47 mV, respectively. The calibration bars refer to all traces.

Table 1. Comparison of the three types of EJP recorded from detrusor smooth muscle of guinea-pig urinary bladder

EJP type	Amplitude (mV)	Latency (ms)	Rise time (ms)	Decay time constant (ms)	n
1	22·4 ± 1·6	26 ± 1	48 ± 3	107 ± 5	48
2	31·8 ± 1·3	45 ± 3	138 ± 12	93 ± 4	48
3	35·7 ± 1·2	28 ± 1	62 ± 3	87 ± 3	63

All values are means ± S.E.M. Amplitude, latency, rise time and decay time constant differed significantly among the three EJP types (one-way analysis of variance, $P < 0{\cdot}01$).

nerve-evoked contractions of guinea-pig and rat urinary bladder are also partially resistant to ω-conotoxin (Maggi *et al.* 1988; De Luca, Li, Rand, Reid, Thaina & Wong-Dusting, 1990). Both single- and two-component EJPs were unaffected by the addition of the muscarinic receptor antagonist atropine ($n = 6$). The effect of desensitization of P_2-purinoceptors with m-ATP was also examined. Perfusion with m-ATP (10 μM) caused a membrane depolarization of 18–28 mV (22·2 ± 1·9 mV; $n = 5$) which was associated with contraction of the tissue. In the continued presence of m-ATP the membrane potential gradually returned to baseline over the next 2 min. Following desensitization of P_2-purinoceptors with m-ATP both types of EJP were abolished (Fig. 4*C–E*).

These results suggest that both single- and two-component EJPs result solely from the stimulation and release of transmitter from post-ganglionic nerve fibres. Both types of response appear to be purinergic. The observation that both types of EJP were inhibited to a similar degree by ω-conotoxin suggests that both rely on a similar mechanism for the release of transmitter from nerve terminals which is only partially dependent upon the influx of Ca^{2+} through Ca_N channels.

Effects of repetitive nerve stimulation on excitatory junction potentials

The effects of repetitive nerve stimulation on the amplitude and time course of both single- and two-component responses was examined. In cells showing single-component (type 1) EJPs, trains of supramaximal stimuli delivered at 0·5 Hz caused a 10% reduction in the amplitude, with little effect on the time course, of the second and subsequent EJPs (Fig. 5*A*; $n = 8$). When trains (0·5 Hz, 30 s) of submaximal stimuli were given, there were small fluctuations in the amplitude of successive EJPs with

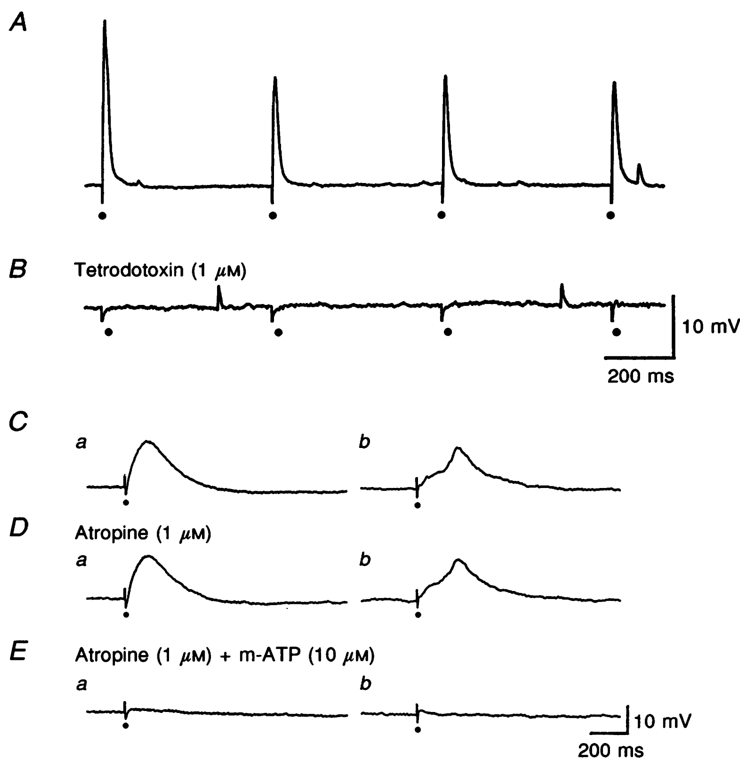

Figure 4. Effect of tetrodotoxin, atropine and m-ATP on responses to field stimulation recorded from detrusor smooth muscle of guinea-pig urinary bladder

The upper two traces show intracellular recordings taken from the same cell before (*A*) and after (*B*) the addition of tetrodotoxin (1 μM) to the physiological saline. In control solution EJPs were evoked by single brief supramaximal stimuli (30 V, 0·5 ms; ●). Following the addition of tetrodotoxin to the perfusion fluid, EJPs were abolished. Note the presence of SEJPs before and after perfusion with tetrodotoxin-containing solution. *C, D* and *E*, the effect of atropine (*D*; 1 μM) and m-ATP (*E*; 10 μM) on type 1 (*a*) and type 2 (*b*) EJPs evoked by single supramaximal stimuli (30 V, 0·5 ms). Atropine (*D*) had no effect on either type of EJP. Both types of EJP were abolished following desensitization of P_2-purinoceptors with m-ATP (*E*). In all records nifedipine (10 μM) was present throughout. The resting membrane potentials were: *A* and *B*, −51 mV; *Ca–Ea*, −41 mV; *Cb–Eb*, −32 mV. The upper calibration bars refer to *A* and *B*; the lower calibration bars refer to *C, D* and *E*.

little change to their time course (Fig. 5*B*). In some cells, when the stimulation strength was reduced to a point at which a first detectable EJP was obtained, not all stimuli in the train were able to evoke EJPs. Presumably this reflects the failure of transmitter to be released from varicosities innervating this or other electrically close cells with each stimulus. Facilitation with low frequency stimulation, as is characteristic of EJPs in other smooth muscle tissues (submucosal arterioles: Hirst, 1977; guinea-pig vas deferens: Burnstock, Holman & Kuriyama, 1964), was never observed.

Similar observations were made using supramaximal strengths of stimulation in cells with type 3 EJPs. EJPs following the initial stimulus were reduced in amplitude with little change to their time course (Fig. 5*C*). However, when either the stimulus strength or pulse width was reduced so as to evoke a two-component EJP, stimulation at a frequency of 0·5 Hz resulted in a marked reduction in the amplitude of the second component of the response (Fig. 5*D*). The first EJP in a train had an amplitude of $26{\cdot}7 \pm 4{\cdot}5$ mV and a time to peak of 346 ± 58 ms. The second EJP had an amplitude of $9{\cdot}0 \pm 2{\cdot}1$ mV and a time to peak of 292 ± 31 ms ($n = 8$). Unlike single-component EJPs, stimulation at stimulus strengths just above threshold never failed to evoke the initial component of two-component EJPs.

The effect of multiple pulses on two-component (type 2) EJPs was further examined in six preparations by increasing the time interval between the first and second stimulus. An example is shown in Fig. 6*A*–*E*. If the second impulse occurred during the latent period, the EJP was increased in amplitude and its latency reduced (Fig. 6*A*). However, if the second impulse coincided with either the rising or falling phase of the second component of the EJP, there was little membrane potential change associated with the second impulse (Fig. 6*B* and *C*). If the second impulse fell after completion of the initial EJP, the second component of the response was either abolished or greatly reduced leaving an underlying membrane depolarization which had a simple time course (Fig. 6*D*). In general a period of at least 30 s between stimuli was required for complete recovery of the EJP.

Taken together, these observations suggest that transmural nerve stimulation of detrusor smooth muscle of the guinea-pig can result in the activation of at least two different membrane conductances. Single-component EJPs, similar to those previously described in urinary bladder (Creed *et al.* 1983), presumably result from the activation of a non-selective cation conductance (Inoue & Brading, 1990). Two-component EJPs may also result from the activation of a non-selective cation conductance, but in addition appear to involve the activation of a second, perhaps voltage-dependent, conductance. It is unclear from the present experiments what the ionic nature of this component of the response is.

Spontaneous excitatory junction potentials

In most cells, as well as evoked potentials, spontaneous depolarizations of the membrane potential (SEJPs) were recorded (see Figs 4 and 7*A*). SEJPs varied in amplitude from less than the recording noise up to 31·4 mV

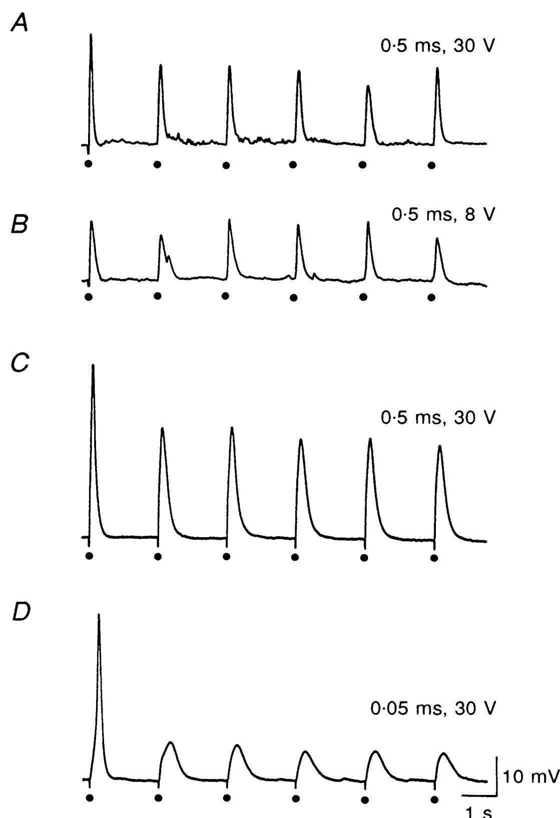

Figure 5. Comparison of the effects of repetitive nerve stimulation on type 1 and type 2 EJPs recorded from detrusor smooth muscle of guinea-pig urinary bladder

A and *B*, type 1 EJPs recorded in response to trains of single brief stimuli (stimulation frequency, 0·5 Hz) at high (*A*; 30 V, 0·5 ms) and low (*B*; 8 V, 0·5 ms) strengths of stimulation. With high strengths of stimulation EJPs are smaller in amplitude following the initial stimulus with little change to their time course. At lower strengths of stimulation there is a small fluctuation in the amplitude of successive EJPs. *C* and *D*, EJPs recorded from a different cell in which a type 3 EJP was recorded. At supramaximal strengths of stimulation (*C*; 30 V, 0·5 ms) there is an approximate 20% reduction in the amplitude of responses following the initial EJP. Stimulation with a shorter pulse width (*D*; 30 V, 0·05 ms) evoked a two-component response, the second component of which was abolished with increasing number of stimuli. The resting membrane potentials were: *A* and *B*, −45 mV; *C* and *D*, −47 mV. Nifedipine (10 μM) was present throughout. The calibration bars refer to all traces.

(13 preparations). In any cell, the amplitude histogram of SEJPs showed a negatively skewed distribution (Fig. 7*B*). Similar results have been obtained for other smooth muscle tissues such as guinea-pig vas deferens (Burnstock & Holman, 1962). Presumably, if urinary bladder smooth muscle is behaving as an electrical syncytium, the variance in amplitude and rise time of SEJPs is related to the distance between the point at which transmitter is released and the recording site (Bennett, 1972). Release at electrically distant points will result in small SEJPs, whereas those resulting from release of transmitter at points close to the recording site will be larger. SEJPs recorded from the three cell types all resembled brief EJPs, which had a simple time course. In general, SEJPs had a briefer time course than neurally evoked simple EJPs (type 1) recorded from the same cell. The falling phase of SEJPs could be described by a single exponential, the time constant of which ranged between 45 and 351 ms (mean, 121 ± 7 ms; $n = 84$). This is similar to that obtained at 25 °C in the rat tail artery (Cassell, McLachlan & Sittiracha, 1988). To compare further the time course of SEJPs in bladder smooth muscle with those in arteries and the vas deferens, recordings were made at 35 °C in three preparations and the time constant of decay of SEJPs over 1·5 mV in amplitude was determined. In general SEJPs decayed with a briefer time course at 35 °C than at 25 °C. The mean time constant of decay of SEJPs at 35 °C for the three preparations was 88 ± 9 ms (range, 42–232 ms; $n = 26$), 46 ± 3 ms (range, 17–75 ms; $n = 20$) and 70 ± 73 ms (range, 26–158 ms; $n = 22$), respectively.

In three bladder preparations the amplitudes of SEJPs were measured before and after addition of tetrodotoxin (1 μM) to the perfusion fluid. The amplitude of SEJPs in control solution ranged between 0·4 and 31·4 mV. In two preparations, after the addition of tetrodotoxin (1 μM), SEJPs larger than 10 mV were abolished (see Fig. 4*B*). This suggests that only small amplitude SEJPs result from the spontaneous release of transmitter from varicosities, whereas large amplitude SEJPs are likely to result from spontaneous activation of nerve branches causing the release of transmitter from multiple sites. In the third preparation, both small and large amplitude SEJPs (range, 0·4–28 mV) persisted in the presence of tetrodotoxin (1 μM). Similar large amplitude SEJPs have been recorded from the guinea-pig vas deferens (Burnstock & Holman, 1962) and arterioles (Hirst & Neild, 1980). However, the

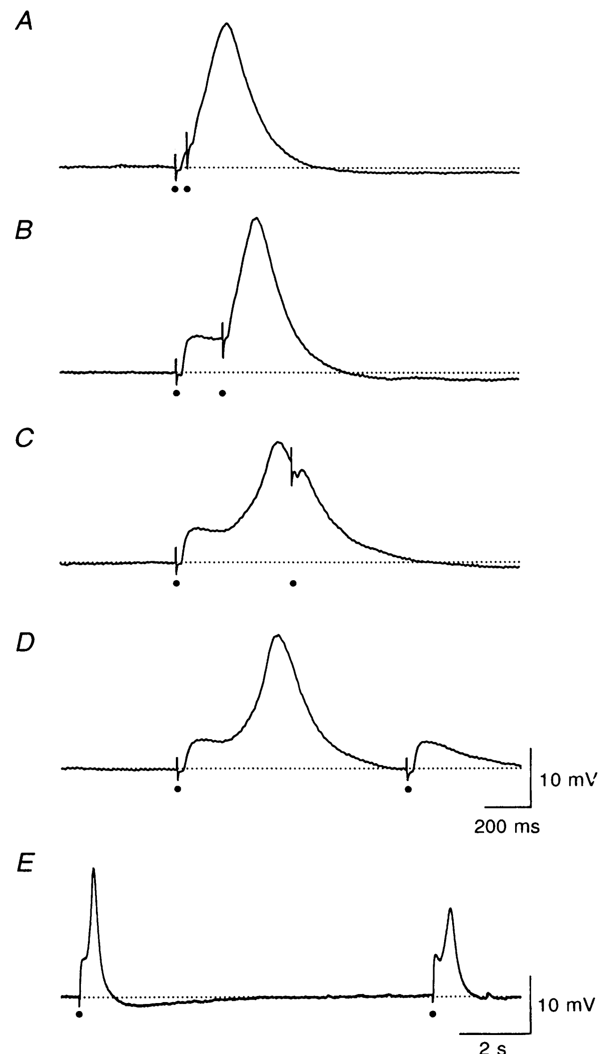

Figure 6. Effect of varying the interpulse interval on type 2 EJPs recorded from detrusor smooth muscle of guinea-pig urinary bladder

A–E, responses evoked by a pair of pulses delivered 0·1 s (*A*), 0·2 s (*B*), 0·5 s (*C*), 1·0 s (*D*) and 10 s (*E*) apart. Note that if the second stimulus is applied before the peak of the initial membrane depolarization the resultant EJP has a rapid onset and consists of a single component. If the second pulse is applied shortly after the peak of the initial membrane depolarization no further depolarization is evoked by the second stimulus (*B*). However the second component of the response has a faster rise time (compare with the response to the first stimulus in *D*). No membrane depolarization is evoked by the second stimulus if it is applied shortly after the peak of the second component of the EJP (*C*). Stimulation after the completion of the initial EJP resulted in depression of the second component of the response (*D* and *E*). *A–E* were all recorded from the same cell in the presence of nifedipine (10 μM). The upper calibration bars refer to *A–D*. The lower calibration bars refer to *E*. The resting membrane potential in all traces was −45 mV.

mechanisms underlying such large membrane depolarizations are unknown.

The observations that SEJPs had briefer time courses than evoked responses and that the SEJP amplitude histograms were negatively skewed, disappearing into the noise, suggest that the urinary bladder, like other smooth muscle, behaves as an electrical syncytium. However, the large variation in both the amplitude and the time course of EJPs recorded from any given preparation suggests that the coupling between cells must be poor. The following series of experiments were undertaken to examine the degree of electrical coupling which exists between cells of guinea-pig urinary bladder smooth muscle.

Electrical properties of urinary bladder smooth muscle

If cells within urinary bladder smooth muscle are electrically well coupled it would be expected that current injected at a point will rapidly pass into neighbouring cells (Jack, Noble & Tsien, 1975). Hence, the membrane potential change induced by current injection into a cell will have a fast time of onset, or decay, and small amplitude. In thirty-two cells from nine preparations the amplitude and time course of membrane potential changes induced by intracellular current injection were examined. Hyperpolarizing current steps of 0·1 nA, 500 ms duration, induced a membrane potential change of 1·2–12·3 mV. The relationship between the amplitude of electrotonic potentials and injected current (−0·4 to 0·2 nA) was of a linear nature (Fig. 8). Cells had input resistances of 65·4 ± 6 MΩ (range, 12–123 MΩ). The time constants of decay of electrotonic potentials ranged between 3 and 23 ms (mean, 11·6 ± 0·9 ms).

The electrical properties of the smooth muscle were further examined using a two microelectrode technique (see Bywater *et al.* 1990). Initially both microelectrodes, placed 40–240 μm apart, were used to record membrane potential. During this time the nerves were stimulated transmurally (0·5 ms, 30 V) and evoked responses were recorded from both cells. One electrode was then switched to pass current and any induced membrane potential change was recorded with the second microelectrode. To determine whether there was any directionality to the coupling between cells, the recording and current passing electrodes were placed in line either along the axis of the muscle bundle (axial) or at right angles to the axis of the muscle bundle (transverse).

In eight of the thirteen pairs of cells examined (from 6 preparations), two electrodes were placed between 20 and 240 μm apart both axially and transversely. In all cells, transmural nerve stimulation (0·5 ms, 30 V) evoked EJPs with variable amplitudes and time courses. Although SEJPs were observed in all cells, in any given pair of cells SEJPs never occurred simultaneously, even when the amplitude of SEJPs was greater than that of the evoked responses (Fig. 9*A*). In these eight pairs of cells, current

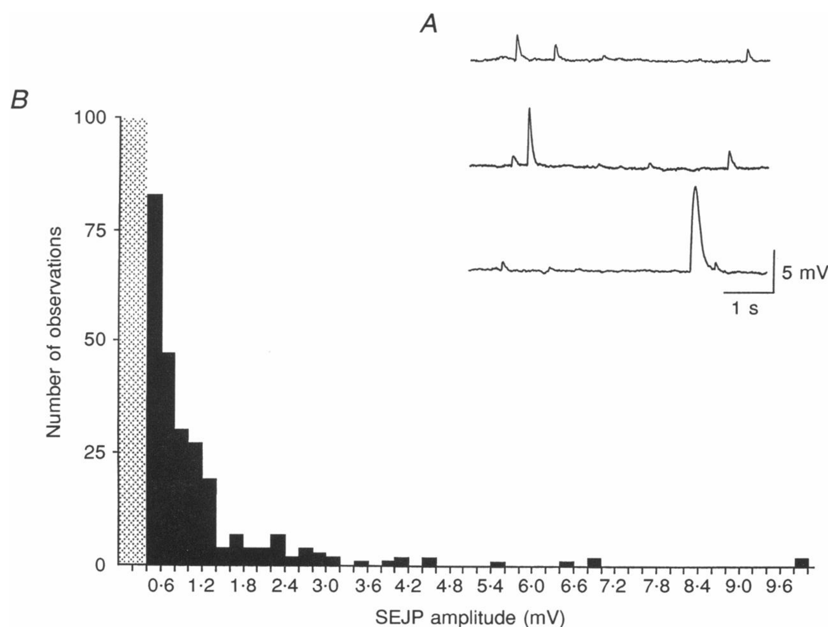

Figure 7. Amplitude–frequency histogram of SEJPs recorded from detrusor smooth muscle of guinea-pig urinary bladder

A, intracellular recordings taken from a detrusor smooth muscle cell in the presence of nifedipine (10 μM). The stable membrane potential is interrupted by SEJPs of variable amplitude. The resting membrane potential of this cell was −45 mV. The calibration bars refer to all three traces. *B*, the resultant amplitude–frequency histogram for SEJPs recorded from this cell. The stippled area represents twice the background noise. It can be seen that the histogram has a skewed distribution with the peak disappearing into the recording noise.

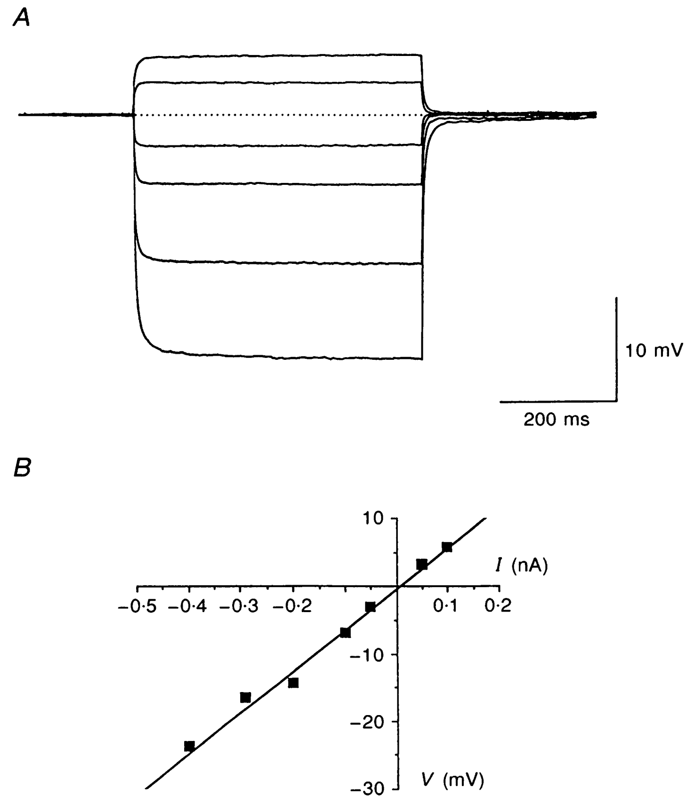

Figure 8. Passive membrane properties of detrusor smooth muscle of guinea-pig urinary bladder

The series of overlaid traces in *A* shows the membrane potential changes recorded following intracellular current injection of +0·1, +0·05, −0·05, −0·1, −0·2, −0·3 and −0·4 nA for 500 ms. *B*, the resultant linear current–voltage relationship for this cell. The cell had a resting membrane potential of −48 mV and an input resistance of 68 MΩ. Nifedipine (10 μM) was present throughout.

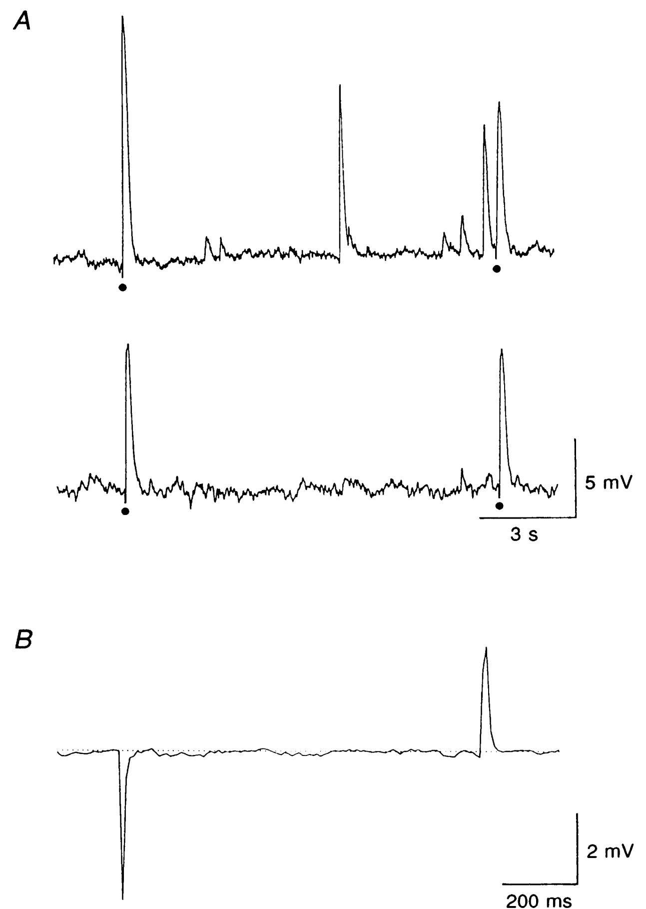

Figure 9. Lack of electrical coupling between two detrusor smooth muscle cells of guinea-pig urinary bladder

A, membrane potential recordings taken simultaneously from two cells of detrusor smooth muscle with the microelectrodes separated by a distance of 40 μm in the axial direction. EJPs evoked by a single supramaximal stimulus (30 V, 0·5 ms; ●) were recorded from both cells. However it can be seen that SEJPs were never simultaneously recorded from the two cells. *B*, the membrane potential recording taken from one cell following injection of a hyperpolarizing current pulse into the second cell (5 nA, 1 s). Note that there is no resultant membrane potential change associated with intracellular current injection. Nifedipine (10 μM) was present throughout. The top calibration bars refer to both traces in *A*. The lower calibration bars refer to *B*. The resting membrane potential of the two cells was −46 and −47 mV.

steps of up to 5 nA failed to induce any membrane potential change in the second cell (Fig. 9*B*). It would therefore appear that coupling in both the axial and transverse direction in this tissue is not extensive.

In one pair of cells, in which the electrodes were placed at a distance of 240 μm transversely, large amplitude SEJPs, which had similar time courses to evoked responses, were recorded simultaneously in both cells. However, injected current steps of up to 5 nA failed to produce any membrane potential change in the second cell, suggesting once again that no coupling existed between the two cells. Presumably, the SEJPs recorded in both cells resulted from the spontaneous activation of a nerve branch causing release of transmitter from multiple sites.

In the remaining four pairs of cells, a varying degree of electrical coupling was observed but only in the axial direction. In three of these pairs of cells, a hyperpolarizing current step of 5 nA, 500 ms, induced a membrane potential change of 0·4 to 2·2 mV which had a slow time of onset and decay (Fig. 10*B*). When both microelectrodes were used to record membrane potential, the majority of SEJPs recorded from the two cells occurred independently of one another. However, occasional simultaneous SEJPs were recorded in both cells (Fig. 10*A*) suggesting that some degree of electrical coupling did exist. It is likely that the majority of SEJPs recorded in one cell result from transmitter release at points electrically too distant from the second cell to give rise to a detectable membrane potential change in that cell.

In one pair of cells, with the microelectrodes placed 60 μm apart axially, all SEJPs were recorded simultaneously from the two cells (Fig. 11*A*). These SEJPs coincided exactly in time, but their amplitudes differed greatly. The observation that simultaneous SEJPs were able to be recorded from these two cells suggests that there is a higher degree of electrical coupling between these cells than the other pairs of cells examined. When a 2 nA, 500 ms hyperpolarizing current pulse was injected into one of these cells, a 9 mV membrane potential change was induced in the second cell (Fig. 11*B*). The time course of onset and decay of this potential change was faster than that of electrotonic potentials recorded from other cells.

DISCUSSION

Transmural nerve stimulation of guinea-pig urinary bladder smooth muscle evoked an EJP which triggered a muscle action potential, so initiating a contraction (Creed *et al.* 1983; Brading & Mostwin, 1989). In the guinea-pig, transmural nerve stimulation with a single impulse evokes EJPs and contractions which are little affected by the muscarinic receptor antagonist atropine, but are abolished after desensitization with m-ATP (Hoyle & Burnstock, 1985; Fujii, 1988; Creed *et al.* 1994). It has therefore been suggested that such responses result predominantly from the release of ATP and the subsequent activation of P_2-purinoceptors. However, in this species, the contribution made by acetylcholine to contractile reponses is thought to increase with an increase in the number and frequency of stimuli delivered to the nerves (Fujii, 1988; Brading & Mostwin, 1989). In the majority of cells, muscle action potentials were abolished by nifedipine, leaving an underlying EJP which had a short latency (< 30 ms) and a

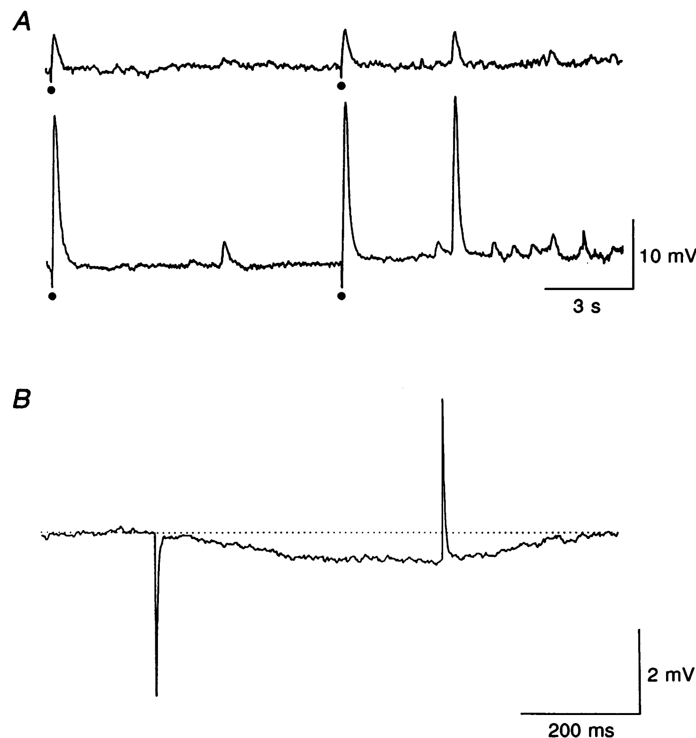

Figure 10. Electrical coupling between two cells of detrusor smooth muscle of the guinea-pig urinary bladder

A, membrane potential recordings taken simultaneously from two cells of detrusor smooth muscle with the microelectrodes separated by a distance of 60 μm in the axial direction. EJPs evoked by a single brief supramaximal stimulus (30 V, 0·5 ms) were recorded in both cells. In general SEJPs recorded from the two cells were asynchronous but occasionally SEJPs occurred synchronously. B, the resultant membrane potential change evoked in one of the cells following injection of hyperpolarizing current (2 nA, 500 ms) into the second cell. Intracellular current injection evoked a small membrane potential change in the second cell which had a slow time of onset and decay. Nifedipine (10 μM) was present throughout. The top calibration bars refer to both traces in *A*. The lower calibration bars refer to *B*. The resting membrane potential for both cells was −46 mV.

total duration of about 400 ms. EJPs with similar time courses have been recorded from guinea-pig, rabbit and pig urinary bladder using the double sucrose-gap technique (Creed *et al.* 1983; Fujii, 1988; Creed, Ito & Katsuyama, 1991). EJPs with short latencies involve the direct activation of receptor-operated ion channels (Hille, 1992). In urinary bladder smooth muscle, applied ATP activates a non-selective cation current which also has a short latency (20 ms; Inoue & Brading, 1990). Thus activation of this current is thought to cause a membrane depolarization and initiate an action potential by opening voltage-dependent Ca^{2+} channels (Mostwin, 1986).

In addition to simple EJPs, EJPs were often recorded which had complex time courses. Frequently a monophasic EJP, resembling those detected in arteries and the vas deferens (Holman, Taylor & Tomita, 1977; Holman & Surprenant, 1979), appeared to initiate a voltage-dependent component which persisted in the presence of organic Ca^{2+} antagonists. Two-component EJPs, which are nifedipine resistant, have also been described in a population of cells from the mouse vas deferens (Holman *et al.* 1977). In these cells, a rapid depolarization could also be evoked by injection of depolarizing current steps. This response persists in the presence of nifedipine but is abolished by tetrodotoxin (Holman, Tonta, Parkington & Coleman, 1995), suggesting that it results from the activation of voltage-dependent Na^+ channels. A similar voltage-dependent Na^+ conductance may be activated in the guinea-pig urinary bladder. However, this idea could not be tested further as in bladder smooth muscle intracellular current injection failed to cause active responses. Alternatively the superimposed component of such EJPs may have resulted from the direct activation of an additional conductance by the neuronal release of transmitter. It is not clear why EJPs with such differing time courses could be recorded from any given preparation, even with closely spaced recording points. Perhaps cells have quite different complements of ion channels in their membranes or cells may receive different patterns of innervation.

Although the rise time of EJPs recorded from guinea-pig urinary bladder was somewhat similar to those recorded from the guinea-pig vas deferens and a number of arteries (Holman, 1970), their rates of decay were much faster; the time constant of decay of EJPs in the bladder was about 100 ms whereas in arteries and the vas deferens they are invariably in excess of 200 ms and often up to 500 ms (Holman *et al.* 1977; Holman & Surprenant, 1979; Cassell *et al.* 1988). In arteries and the vas deferens the time constant of decay of EJPs reflects the passive membrane time constants of those tissues (Bywater & Taylor, 1980; see Hirst & Edwards, 1988). If the same considerations are applied to bladder smooth muscle, these observations suggest that the membrane time constant of this tissue is

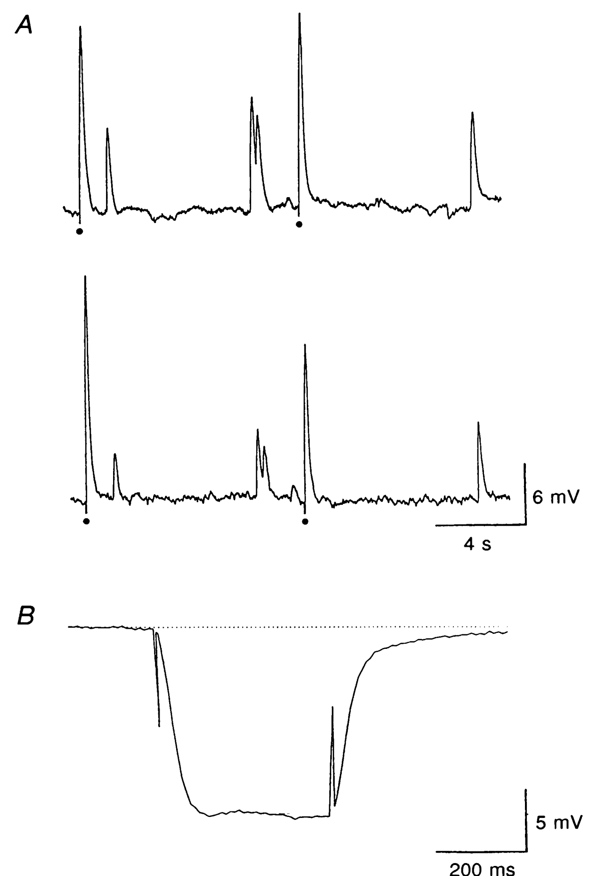

Figure 11. Electrical coupling between two cells of detrusor smooth muscle of the guinea-pig urinary bladder

A, membrane potential recordings taken simultaneously from two cells of detrusor smooth muscle with the microelectrodes separated by a distance of 60 μm in the axial direction. EJPs evoked by a single supramaximal stimulus (30 V, 0·5 ms) were recorded in both cells. All SEJPs were recorded synchronously from both cells suggesting that the two cells were electrically coupled. *B*, the resultant membrane potential change evoked in one cell after injection of hyperpolarizing current (2 nA, 400 ms) into the second cell. Nifedipine (10 μM) was present throughout. The top calibration bars refer to both traces in *A*. The lower calibration bars refer to *B*. The resting membrane potential in both cells was −47 mV.

quite brief, perhaps more similar to that determined for intestinal smooth muscle (Cousins, Edwards, Hirst & Wendt, 1993).

SEJPs were recorded from most cells of the urinary bladder smooth muscle. These varied greatly in both amplitude and time course, with most SEJPs having small amplitudes that were difficult to resolve from the recording noise. Similar observations have been made in other smooth muscle tissues, including guinea-pig and mouse vas deferens (Burnstock & Holman, 1962; Brock & Cunnane, 1988) and many arteries (Kuriyama & Suzuki, 1981; Cassell *et al.* 1988). In all of these tissues, SEJPs are thought to result from the spontaneous release of transmitter from varicosities and the subsequent activation of ATP-gated non-selective cation channels (Benham & Tsien, 1987; Friel, 1988). However the durations of SEJPs in bladder were more prolonged (decay time constant, 50–90 ms at 35 °C) than those observed in either arteries (decay time constant, 20–30 ms; Cassell *et al.* 1988) or the vas deferens (decay time constant, 30–40 ms; Burnstock & Holman, 1962; Brock & Cunnane, 1988). This suggests that either the time course of action of transmitter at this junction is longer than that observed in other tissues, or that the electrical properties of urinary bladder are different. There may be a difference in the degree of electrical coupling between cells within urinary bladder smooth muscle. In submucosal arterioles, even though the time course of the underlying current is similar to that described in both arteries and the vas deferens (Finkel, Hirst & Van Helden, 1984), the time constant for the decay of SEJPs approaches that of an EJP (Hirst, 1977). This has been attributed to a difference in the anatomy of arterioles compared with other smooth muscle tissues; arterioles are thin walled and behave like one-dimensional cables (Hirst & Edwards, 1988). Other tissues are thicker and behave like two- or three-dimensional cables (Bennett, 1972). Thus, if coupling within urinary bladder smooth muscle was poor, the time constant for the decay of SEJPs in this tissue would be expected to be closer to that of an EJP.

A number of observations suggest that the pattern of electrical coupling in bladder differs from that in most other smooth muscle tissues. Firstly, EJPs varied greatly in both their amplitude and time course even when recorded from cells at similar distances from the stimulating electrodes. In an electrical syncytium, where there are no electrical discontinuities between either single cells or bundles of cells, transmural nerve stimulation causes the synchronous release of transmitter at many sites and results in the tissue being isopotential during much of the EJP. Thus, EJPs occurring in different cells have similar amplitudes and time courses when recorded at a set distance from the point of stimulation. Secondly, the observation that as the strength of field stimulation was reduced the amplitude of EJPs was decreased in two or three discrete steps, rather than gradually, also suggests that electrical coupling within detrusor smooth muscle is different from that of other smooth muscle tissues. In well coupled densely innervated tissues, such as the guinea-pig vas deferens, decreasing the strength of stimulation results in a graded reduction in the size of the response to nerve stimulation (Holman *et al.* 1977). However, in tissues which are well coupled but have a sparse innervation, such as arterioles, the decrease in amplitude of EJPs with stimulus strength occurs in discrete steps (Hirst, 1977). This is also the case for tissues, such as the mouse vas deferens (Holman *et al.* 1977), which have a dense innervation but are not well coupled. Therefore, as in the mouse vas deferens, electrical coupling between cells of the urinary bladder may not be extensive.

In the present study the syncytial properties of guinea-pig urinary bladder smooth muscle were examined in two ways. Initially the tissue was impaled with one electrode and the input resistance was determined. The input resistance and time constant of decay of membrane potential changes induced by point current injection were approximately 65 MΩ and 12 ms, respectively. These values are similar to those obtained for the mouse vas deferens (input resistance, 20–200 MΩ; decay time constant, 3–25 ms; Holman *et al.* 1977). However, in the guinea-pig vas deferens, a tissue thought to be electrically well coupled, the majority of cells have lower input resistances (10–30 MΩ) and briefer decay time constants (1–5 ms; Holman *et al.* 1977). The syncytial properties of guinea-pig urinary bladder smooth muscle were also examined by impaling the tissue with two microelectrodes; one electrode was used to pass current and the other to record the resultant changes in membrane potential induced in the second cell. In the majority of cases, when recordings were made from points close to the site of current injection, there was no evidence for the existence of electrical coupling between cells; no resultant electrotonic potentials were recorded and all SEJPs recorded from the two cells were asynchronous. In only a small proportion of recordings, when electrodes were placed between 20 and 60 μm apart in the axial direction, were electrotonic potentials and synchronous SEJPs observed. In such instances the electrotonic potentials evoked by intracellular current injection were small in amplitude when compared with other syncytial tissues thought to be electrically well coupled (submucosal arterioles: Hirst & Neild, 1978; ileum: Cousins, Edwards, Hirst & Wendt, 1993; toad sinus venosus: Bywater *et al.* 1990). Taken together these results suggest that the electrical coupling between cells of the guinea-pig urinary bladder is less extensive than other syncytial tissues so far examined. Although all individual bladder cells are electrically connected to other nearby cells to form discrete bundles, it seems most likely that there is poor coupling between bundles.

Given that there appears to be such poor coupling between muscle bundles of the urinary bladder, it might be expected that to achieve synchronous contraction of the entire

bladder wall, muscle bundles either receive an extremely dense innervation, or the probability of transmitter release at this neuroeffector junction is high when compared with other smooth muscle tissues (Hirst & Neild, 1980; Brock & Cunnane, 1988). This latter possibility seems unlikely because in some cells, during trains of stimuli, there was an occasional fluctuation in the rise time and amplitude of EJPs (N. J. Bramich and A. F. Brading, unpublished observation). Such observations have also been made in the guinea-pig vas deferens (Burnstock & Holman, 1962) and have been attributed to the intermittent release of transmitter from varicosities: EJPs with faster rise times resulting from the release of transmitter at a point close to the recording electrode (Burnstock & Holman, 1962; Hirst & Neild, 1980). Alternatively, the density of innervation in this tissue may be high. Ultrastructural studies of detrusor smooth muscle of the rat have shown that muscle cells receive an extensive innervation (Gabella & Uvelius, 1990; Gabella, 1995). Also, in human detrusor smooth muscle, it has been estimated that smooth muscle cells are each associated with three to four varicosities (Daniel, Cowan & Daniel, 1983). Such a dense innervation may therefore allow the co-ordination of contraction of the entire dome of the urinary bladder.

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