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Electrical and mechanical responses produced by nerve stimulation in detrusor smooth muscle of the guinea-pig

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Abstract

In smooth muscles of the guinea-pig bladder, intramural nerve stimulation generated an excitatory junctional potential (e.j.p.), action potential and twitch contraction. Nicardipine inhibited the action potential but not the e.j.p. The e.j.p. amplitude was reduced by suramin, or desensitization of the ATP receptor with receptor agonists. The amplitude of the twitch contraction was reduced by atropine, and the remainder was blocked by nicardipine. In the presence of maximally effective concentrations of atropine, the threshold concentration of acetylcholine required to produce contraction was about 10^{-7} M, whereas acetylcholine concentrations greater than 10^{-6} M were required to cause depolarization. It is concluded that nerve stimulation releases acetylcholine and ATP, and the former produces contraction without change in the membrane potential, while the latter generates the e.j.p. which triggers an action potential and thus elicits contractions.

Keywords: Detrusor smooth muscle; Electrical response; Excitatory junctional potential; (Contraction)

1. Introduction

In most mammalian urinary bladders, contractile responses of isolated smooth muscle elicited by intramural nerve stimulation are mediated by atropine-sensitive cholinergic and atropine-resistant non-adrenergic, non-cholinergic (NANC) nerves (Sibley, 1984). In contrast, the human urinary bladder receives excitatory cholinergic innervation only, and no atropine-resistant contraction seems to exist under physiological conditions (Sibley, 1984; Kinder and Mundy, 1985), although the atropine-resistant contractions appear in pathological conditions (Sjögren et al., 1982; Husted et al., 1983; Palea et al., 1993).

Stimulation of the intramural nerves innervating the smooth muscle of the urinary bladder has been shown to produce an excitatory junction potential (e.j.p.), resulting in a spike potential (Creed et al., 1983; Fujii, 1988; Brading and Mostwin, 1989) and a slow depolarizing response following the e.j.p. (Creed et al., 1983; Fujii, 1988). Blockade by atropine of the slow depolarization indicates that this potential is produced by acetylcholine (Creed et al., 1983). In many animal species, the e.j.p.s are resistant to sympathetic and parasympathetic blocking agents, and thus the potentials are considered non-adrenergic, non-cholinergic (NANC) in nature (Creed et al., 1983). In the guinea-pig bladder, the e.j.p. can be mimicked by local application of ATP (Burnstock et al., 1972). Release of a sufficient amount of ATP for the generation of the e.j.p.s from intramural nerves has also been reported (Burnstock et al., 1978). Using double sucrose gap methods, desensitization of P_2 purinoceptors by α,β-methylene ATP strongly inhibits the e.j.p. (Kasakov and Burnstock, 1983; Creed et al., 1983; Fujii, 1988; Brading and Mostwin, 1989). In enzymatically isolated single cells of the guinea-pig bladder, ATP induces increases in ionic conductance by opening of non-selective cation channels (Inoue and Brading, 1990; Schneider et al., 1991; Nakayama, 1993). These findings suggest that the e.j.p. is produced by ATP. Suramin, a P_2 purinoceptor antagonist, inhibits contractile responses to α,β-methylene ATP, and inhibits responses to stimulation of intramural purinergic nerves (Hoyle et al., 1990). Recently, the double-sucrose gap method has been used to demonstrate the inhibitory effects of suramin on the e.j.p. in guinea-pig bladder (Creed et al., 1994).

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The electrical components related to the nerve-mediated contractions of bladder smooth muscle are, therefore, the e.j.p., slow depolarization and spike potential. The slow depolarization and spike potential are sensitive to atropine and Ca^{2+} antagonists, respectively (Creed et al., 1983). Application of these pharmacological tools allows the measurement of the contribution of the e.j.p. in nerve-mediated contractions.

In urethral smooth muscle preparations of many mammalian species including the human, electrical stimulation elicits relaxation which is mediated by nitric oxide (NO) (Andersson et al., 1992). Furthermore, it has been demonstrated that inhibition of the L-arginine/nitric oxide pathway leads to bladder hyperactivity and decreases bladder capacity in vivo in the rat (Persson et al., 1992). Thus continuous activity in this pathway is presumed to be one of the factors for maintaining bladder capacity. In pre-contracted isolated detrusor smooth muscle of the rat, nerve-mediated relaxations are absent and nerve-induced contractions are unaffected by *N*-nitro-L-arginine methylester or L-arginine, while NO or NO producers such as 3-morpholinosydnonimine and sodium nitroprusside cause small relaxations (Persson et al., 1992). Therefore, the functional role of NO in detrusor smooth muscle is questionable.

The present study was carried out to determine the transmitter(s) responsible for the e.j.p. and the possible contribution of this potential to nerve-mediated contraction in isolated detrusor smooth muscle from the guinea-pig bladder. The possible involvement of NO in the electrical responses of the smooth muscle membrane to nerve stimulation was also examined using intracellular microelectrodes.

2. Material and methods

2.1. Preparations

Male albino guinea-pigs, weighing 200–250 g, were stunned and bled. The bladder was excised and opened in Krebs solution. The mucosal layer was removed, and rectangular strips 2 mm in width and 10 mm long were prepared.

2.2. Microelectrode experiments

Conventional microelectrode techniques were used to record the electrical responses from smooth muscle cells. Glass capillary microelectrodes (outer diameter, 1.2 mm) filled with 3 M KCl and with tip resistance of 40–80 MΩ were used. The isolated bladder preparation was mounted on a silicone-rubber plate, with the mucosal side uppermost. Transmural nerve stimulation was applied using point stimulation, namely, a silver wire electrode (diameter, 0.5 mm) coated with enamel except at the very tip was gently attached to one end of the tissue segment, and a silver plate (2 × 5 mm) coated with AgCl was located on the opposite side of recording chamber. Current pulses of 50–100 μs duration and 10–100 V intensity were applied between these two silver electrodes, using an electric stimulator (SEN-3103, Nihon-Kohden, Japan). The responses were displayed on a cathode-ray oscilloscope (SS-7602, Iwatsu, Japan) and recorded on a pen recorder (Recticorder, RJG-4124, Nihon-Kohden, Japan).

2.3. Contraction experiments

Muscle strips 2 mm wide and 10 mm long were tied at both ends with cotton threads, with one end connected to a mechano-transducer (FD pickup, TB-612T, Nihon-Kohden, Japan) and the other to the bottom of the recording chamber (cylindrical in shape, 8 mm diameter, 15 mm high, volume about 1.5 ml). The tissue was perfused with aerated warmed (35°C) Krebs solution supplied at a constant flow rate of 3 ml/min, using a peristaltic pump (PO-1, Taiyo-Rikakikai, Tokyo, Japan). Mechanical responses of the muscle strips were measured isometrically, under a resting tension of about 50 mg. A pair of silver plates (2 mm width, 15 mm long) were fixed vertically to both sides of the recording chamber allowing electrical stimulation to be applied transmurally to the muscle.

2.4. Solutions

The ionic composition of the Krebs solution was as follows (in mM); Na^+ 134, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 137, glucose 15.5. The solution was aerated with O_2 containing 5% CO_2, and the pH of the solution was maintained at 7.3–7.4.

2.5. Drugs

Drugs used were adenosine 5′-triphosphate disodium salt (ATP), α,β-methylene-ATP, acetylcholine chloride, atropine sulfate, guanethidine sulfate, tetrodotoxin, nicardipine, suramin, *N*-nitro-L-arginine, L-arginine and sodium nitroprusside.

2.6. Statistics

The recorded values were expressed as the means ± standard deviation (S.D.). Statistical significance of the values was estimated using the paired and unpaired Student's *t*-test, and probabilities of less than 5% were considered significant.

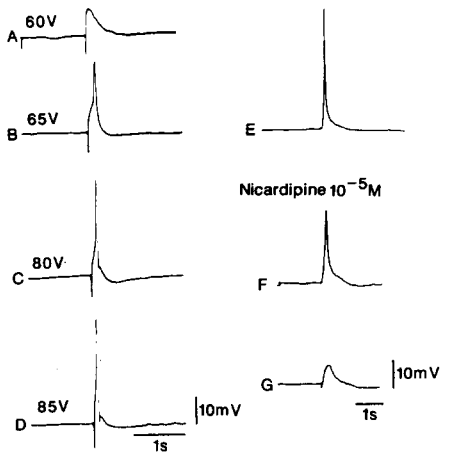

Fig. 1. Electrical responses elicited by transmural nerve stimulation, as recorded from guinea-pig detrusor smooth muscle. A–D: Electrical responses elicited by increasing intensity of stimuli (A, 60 V; B, 65 V; C, 80 V; D, 85 V) in a single cell (pulse duration, 50 μs). Membrane potential, −47 mV. E–G: Effects of nicardipine (10^{-5} M) on electrical responses elicited by nerve stimulation. Responses were recorded before (E) and after application of nicardipine (F, 5 min; G, 15 min). Stimuli: 50 μs duration, 100 V intensity. Recordings were all from the same cell, with a membrane potential of −43 mV.

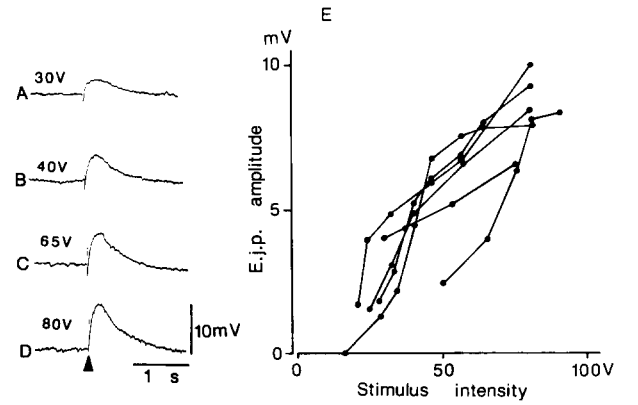

Fig. 2. Electrical responses elicited by nerve stimulation in smooth muscle cells of the guinea-pig detrusor. All the recordings were obtained in the presence of 10^{-5} M nicardipine. Transmural nerve stimulation of increasing intensity (A, 30 V; B, 40 V; C, 65 V; D, 80 V) was applied. Recordings are all from the same cell. The membrane potential was −54 mV. E: The relationship between intensity of stimulation and amplitude of the e.j.p. recorded from six different cells. Nicardipine 10^{-5} M was present throughout. Stimulus duration: 50 μs.

3. Results

3.1. Electrical responses of the membrane to nerve stimulation

The resting membrane potential of smooth muscle cells in the guinea-pig detrusor ranged between −30 and −50 mV (mean, -39.8 ± 4.4 mV, $n = 785$ cells). In most cells studied, the membranes were spontaneously active with spike discharges at various frequencies.

Transmural application of brief electrical pulses (50–100 μs duration, 20–30 V intensity) evoked depolarizing responses which consisted of fast rising and slow falling phases, with the peak amplitude ranging between 1–10 mV (Fig. 1A). The amplitude of the peak potential was increased with increase in intensity of stimulation with larger responses generating a spike potential (Fig. 1B–D).

Application of 10^{-5} M nicardipine gradually blocked the spike component to reveal underlying depolarizing responses (Fig. 1E–G). The amplitude of this depolarizing response increased with the intensity of stimulation with peak amplitudes of between 8–12 mV (Fig. 2). Nicardipine did not alter the resting membrane potential.

The effects of suramin, nicardipine, atropine and tetrodotoxin on the depolarizing responses elicited by electrical stimulation are shown in Fig. 3. The depolarizing responses elicited by weak stimuli remained unaltered by application of 10^{-5} M nicardipine or 10^{-6} M atropine for over 1 h (Fig. 3A: a,b and c,d). In all of the three tissues studied, these depolarizing responses also remained unaltered by application of 5×10^{-6} M guanethidine for over 1 h. The responses were, however, inhibited reversibly by application of 10^{-6} M tetrodotoxin (Fig. 3A: e,f). These results indicate that the depolarizing response was an excitatory junction potential (e.j.p.) elicited as a result of excitation of intramural non-adrenergic non-cholinergic (NANC) nerves.

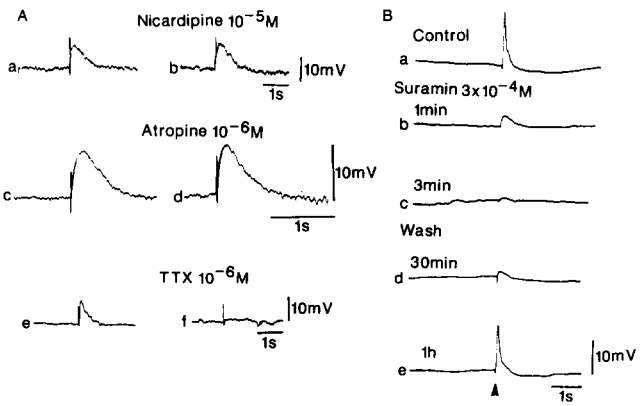

Fig. 3. The effects of nicardipine 10^{-5} M (A: a,b), atropine 10^{-6} M (A: c,d), tetrodotoxin 10^{-6} M (A: e,f) and suramin 3×10^{-4} M (B: a–e) on the e.j.p.s evoked in smooth muscle of the guinea-pig bladder. Nerve stimulation: 50 μs duration, 30–50 V intensity. The e.j.p.s in each series were recorded from different tissues. Recordings shown in panel B were made in the presence of 10^{-5} M nicardipine. All the responses shown in panel B were recorded from the same cell.

3.2. Nature and properties of the e.j.p.s

Because of the absence of any detectable effect of nicardipine on the e.j.p. and membrane potential (Fig. 3A: b), the properties of the e.j.p. were investigated further in the presence of nicardipine which abolished induced and spontaneous spike activity and contraction. The use of nicardipine allowed measurement of the e.j.p.s and also, by preventing contractions, made it easier to record from the tissue.

The effects of suramin on the e.j.p. are shown in Fig. 3B, the objective being to determine the possible involvement of P_2 purinoceptors in the generation of this potential. Suramin (3×10^{-4} M) inhibited the e.j.p., within 3 min, with no detectable change in the membrane potential. The effect of suramin was reversible, and the recovery required over 30 min washing. Suramin (3×10^{-4} M) also completely inhibited the depolarization produced by ATP (10^{-3} M) or α,β-methylene ATP (3×10^{-6} M) in all of the three tissues studied.

Attempts were made to observe the effects of ATP and α,β-methylene ATP on the e.j.p. in the detrusor muscle. The experiments were carried out to compare the e.j.p.s evoked before and after desensitizing the postjunctional receptors for ATP. The extent of desensitization of the receptors was monitored by the change in membrane potential upon continued application of purinoceptor agonist with desensitization considered complete when the membrane potential returned to its resting level. Exogenously applied ATP (10^{-3} M) depolarized the membrane by about 12 mV, and then the membrane repolarized gradually with time, to reach the resting potential level in about 5 min (Fig. 4A: a). Nerve stimulation was applied to evoke the e.j.p. before application of ATP (Fig. 4A: b), and after repolarization to the resting level in the maintained presence of ATP (Fig. 4A: c). After ATP desensitization, e.j.p.s were markedly smaller in amplitude (10–20% of the control). Application of 3×10^{-6} M α,β-methylene ATP produced a transient depolarization, the peak amplitude being about 18 mV from the resting potential and the duration about 2–4 min (Fig. 4B: a). The e.j.p.s evoked after repolarization to the resting level in the presence of α,β-methylene ATP (4–5 min after agonist application) were significantly smaller than those evoked before application of α,β-methylene ATP (Fig. 4B: b,c).

Application of acetylcholine induced a depolarization which was sustained for up to 10 min (Fig. 4C), and was blocked by 10^{-6} M atropine (as observed in all of four tissues studied).

The concentration-dependent increase in the peak amplitude of the depolarization in response to either acetylcholine or ATP is summarized in Fig. 4D. ATP was more potent than acetylcholine to depolarize the membrane, with 10^{-3} M ATP causing depolarization of about 12 mV, this value being comparable to the peak amplitude of the e.j.p.s.

Application of sodium nitroprusside (10^{-5}–10^{-4} M), *N*-nitro-L-arginine (10^{-5}–10^{-4} M) or L-arginine (10^{-3} M) for over 10 min caused no significant change in membrane potential or in the e.j.p. in each of three tissues studied.

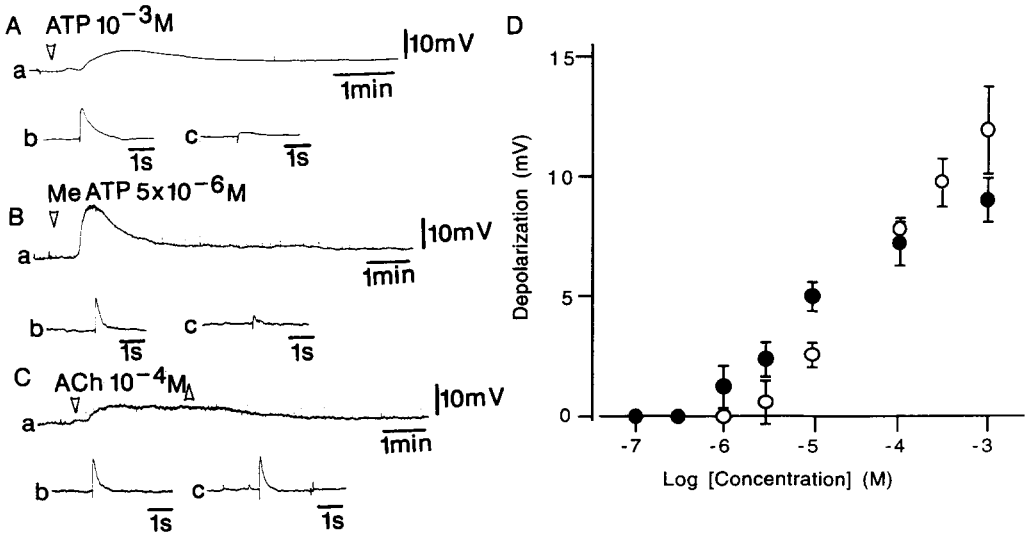

Fig. 4. The effects of ATP, α,β-methylene ATP or acetylcholine on the electrical responses of smooth muscle cells in the guinea-pig bladder. Muscles were exposed to 10^{-3} M ATP (A) or 3×10^{-6} M α,β-methylene ATP (MeATP) (B), and the e.j.p.s were evoked before (b) and after desensitization of purinoceptors with these agents (c). The effects of 10^{-4} M acetylcholine (ACh) on the membrane potential of the guinea-pig bladder are shown in C. A, B, and C were recorded from different tissues (membrane potential: A, −40 mV; B, −43 mV; C, −42 mV). Nerve stimulation of 50 μs duration and 30–50 V intensity was used. All the recordings were made in the presence of 10^{-5} M nicardipine. D: The depolarizations produced by acetylcholine (●) or ATP (○) in smooth muscles of the guinea-pig detrusor. Mean ± S.D. (n = 5–14).

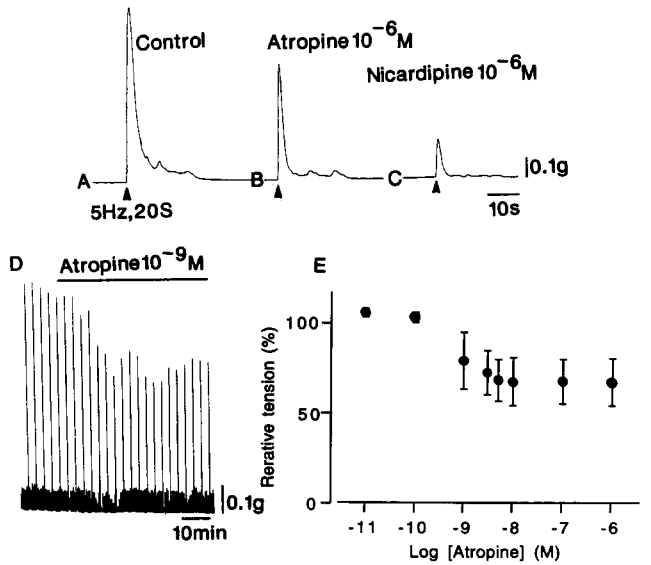

Fig. 5. The effects of atropine and nicardipine on contractions produced by transmural nerve stimulation in smooth muscle of the guinea-pig bladder. Transmural nerve stimulation (5 Hz for 20 s) was applied at the arrow, before (A) and after application of 10^{-6} M atropine for 10 min (B) and additional application of 10^{-6} M nicardipine for 30 min (C). A–C were recorded from the same tissue. D: Muscles were contracted every 3 min by transmural nerve stimulation (10 Hz for 1 s), while atropine (10^{-9} M) was continuously applied as shown by the bar. E: The relationship between the concentration of atropine and the tension produced by nerve stimulation. The tension relative to that before application of atropine is shown as the mean ± S.D. ($n = 10$–12).

3.3. Mechanical responses of bladder smooth muscles

Detrusor smooth muscle was spontaneously active, showing irregular amplitudes of contraction with such activity continued over 6 h. Application of transmural electrical stimulation produced a phasic contraction. The contraction produced by electrical stimulation, but not spontaneous activity, was abolished reversibly by application of tetrodotoxin (10^{-6} M), suggesting that this contraction was produced by stimulation of intramural nerves (ten observations). The effects of atropine and nicardipine on nerve-induced contractions are shown in Fig. 5. Stimulation of nerves for 20 s at 5 Hz produced a contraction with a transient phasic component followed by a small sustained component (Fig. 5A). Atropine (10^{-6} M) reduced the phasic and tonic components of the contractions by about 20–30% (Fig. 5B). Additional application of 10^{-6} M nicardipine reduced the phasic component to 10–20% of the control and abolished the sustained component (Fig. 5C). The residual phasic component was inhibited completely by application of 10^{-5} M nicardipine in all five tissues studied.

The amplitude of contractions produced by nerve stimulation was reduced by atropine in a concentration-dependent manner, and reached a steady amplitude of about 60% of the control at 10^{-8} M (Fig. 5D and E). In order to estimate the concentration of nerve-released acetylcholine acting on the postsynaptic receptors, contractile responses to bath applied acetylcholine were studied in the presence of 10^{-8} M atropine, the minimum concentration that completely suppressed neurogenic cholinergic responses. In these conditions the threshold concentration of acetylcholine required to produce contraction was $(2.5 \pm 1.9) \times 10^{-7}$ M ($n = 7$), implying that the concentration of nerve released acetylcholine achieved was slightly less than this.

4. Discussion

The present experiments showed that transmural nerve stimulation in the detrusor smooth muscle of the guinea-pig causes an e.j.p., resulting in spike potential and contraction. The spike potential is produced mainly by Ca^{2+} influx, since nicardipine, a Ca^{2+} channel antagonist, inhibited this potential. The e.j.p. was non-adrenergic non-cholinergic (NANC) in nature as the potential was resistant to agents which block adrenergic (guanethidine) or cholinergic (atropine) transmission. The e.j.p. was inhibited by either suramin, a P_2 purinoceptor antagonist (Hoyle et al., 1990; Creed et al., 1994), or by desensitization of ATP receptors upon long exposure of muscles to ATP or α,β-methylene ATP (Kasakov and Burnstock, 1983; Creed et al., 1983; Fujii, 1988; Brading and Mostwin, 1989). The results are therefore in agreement with the idea that the e.j.p. is produced by ATP (Fujii, 1988; Brading and Mostwin, 1989). However, the results show that acetylcholine is also involved in neuromuscular transmission, since atropine reduced the contractions produced by nerve stimulation by about 40%.

In the present experiments, the estimated concentration of acetylcholine involved in neuromuscular transmission was about 10^{-7} M. This is about 10 times lower than that required to depolarize the smooth muscle membrane (about 10^{-6} M). In smooth muscles of the rabbit bladder, the electrical responses of the membrane to nerve stimulation are an e.j.p. and following slow depolarization, the latter being produced by activation of atropine-sensitive muscarinic receptors (Creed et al., 1983). In the guinea-pig, the dissociation by atropine of the electrical and mechanical responses induced by nerve stimulation indicate that the concentration of acetylcholine released is below the threshold for depolarization of the postjunctional membrane. In addition, it is known that muscarinic receptor stimulation increases the production of inositol 1,4,5-trisphosphate (IP_3) and induces smooth muscle contraction through release of Ca^{2+} from intracellular Ca^{2+}

stores (Mostwin, 1985; Iacovou et al., 1990; Creed et al., 1992). Thus, as reported by Fujii (1988), acetylcholine seems to contribute to the nerve-mediated contractions by pharmaco-mechanical coupling (Somlyo and Somlyo, 1968).

Inhibition by nicardipine of the action potentials and the mechanical responses produced by nerve stimulation indicates that the atropine-resistant component of contraction may result from activation of voltage-sensitive Ca^{2+} channels (Katsuragi et al., 1990). This component represents about 60% of the nerve-mediated contraction. The finding that nicardipine inhibited the spike potential and the contraction (in the presence of atropine) but not the e.j.p. indicates that the junctional potential itself does not contribute directly to the generation of contraction.

In pre-contracted urethral smooth muscle preparations, the NANC relaxation in response to transmural nerve stimulation is mediated by NO (Andersson et al., 1992), but the contribution of NO in detrusor smooth muscle remains uncertain (Persson et al., 1992). We found that, in the guinea-pig bladder, *N*-nitro-L-arginine and L-arginine caused no detectable change in either membrane potential or e.j.p., and this was also the case for sodium nitroprusside. These results suggest that there is no involvement of NO in the electrical responses of bladder smooth muscle of the guinea-pig to transmural nerve stimulation.

In enzymatically isolated single cells of the guinea-pig bladder, ATP depolarizes the membrane (Schneider et al., 1991). Therefore, ATP could increase $[Ca^{2+}]_i$ by Ca^{2+} influx through ATP-gated non-selective cation channels and voltage-dependent Ca^{2+} channels. Schneider et al. (1991) concluded that the $[Ca^{2+}]_i$ elevation induced by ATP is mainly due to Ca^{2+} influx through the ATP-gated channel which is insensitive to Ca^{2+} channel blockers, and the contribution of Ca^{2+} influx through the voltage-dependent Ca^{2+} channels is less important. However, contradictory data have been presented by Katsuragi et al. (1990) who found that application of Ca^{2+} channel antagonists inhibited ATP-induced contractions in smooth muscle of the guinea-pig bladder. The present experiments showed that the contractions produced by nerve stimulation were completely blocked by nicardipine (atropine present) in concentrations which inhibited the spike potential but not the e.j.p.s. Thus, the e.j.p. itself does not cause contraction, indicating that there is either insufficient Ca^{2+} entry during the e.j.p. or that the junctional channels are inherently different from those studied in dispersed cells (e.g. Schneider et al., 1991).

In normal human bladder, transmural nerve stimulation only elicits an atropine-sensitive contraction and there is little if any contribution of NANC excitatory nerves (Sjögren et al., 1982; Husted et al., 1983; Sibley, 1984; Kinder and Mundy, 1985). In tissues isolated from patients with a functional bladder disturbance, however, nerve stimulation elicits an atropine-resistant contraction (Sjögren et al., 1982; Husted et al., 1983; Palea et al., 1993) and this is inhibited by desensitization of the postjunctional ATP receptors with ATP (Husted et al., 1983). Luheshi and Zar (1990) demonstrated the presence of non-cholinergic motor transmission in human bladder. However, it is uncertain that their results reveal a common property of normal human bladder because their preparations were obtained from patients with lower urinary tract disorders. Postjunctional supersensitivity to transmitter substances is often seen in smooth muscle from bladders with outlet obstruction (Speakman et al., 1987), and preparations obtained from hypertrophied bladder are more sensitive to ATP than those from the normal preparations (Husted et al., 1983). This is one possible explanation for the appearance of NANC components in the contraction. Recently, it has been shown that enzymatically isolated single cells from functionally normal human bladder exhibit an ATP-induced inward current (Inoue and Brading, 1991) and this probably underlies the ATP-induced contraction observed in intact tissues (Husted et al., 1983). Therefore, further investigation of the effects of ATP on human bladder smooth muscle, especially at the neuromuscular junction, in both physiological and pathological conditions is required.

It is concluded that, in smooth muscles of the guinea-pig bladder, nerve stimulation-mediated contractions consist of cholinergic and NANC components. The cholinergic component is produced by acetylcholine in concentrations below the threshold required to depolarize the postjunctional membrane, while the NANC component is produced by e.j.p.-generated spike potentials. The e.j.p. is likely to be produced by ATP, while the spike potential is produced by activation of voltage-sensitive Ca^{2+} channels.

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