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ELECTRICAL AND MECHANICAL ACTIVITY OF THE ISOLATED LOWER URINARY TRACT OF THE GUINEA-PIG

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1 Strips of urethra taken from guinea-pigs contracted in response to acetylcholine, noradrenaline (via α-adrenoceptors) and 5-hydroxytryptamine, and were relaxed by adenosine triphosphate (ATP) if the tone was raised. Isoprenaline produced relaxation of bladder strips (via β-adrenoceptors) whereas ATP caused contraction.

2 Atropine completely blocked all responses to acetylcholine; quinidine failed to block ATP responses selectively; methysergide blocked responses of the urethra but not the bladder to 5-hydroxytryptamine.

3 Spontaneous electrical activity was recorded with intracellular microelectrodes from all regions: in the urethra infrequent bursts of spikes occurred at 1–7 min intervals; regular spikes at 6–30/min were recorded from the detrusor muscle. In the bladder base, bursts of spikes were superimposed on the regular pattern.

4 Bursts of spikes in the urethra were initiated by noradrenaline, phenylephrine or acetylcholine and inhibited by ATP; regular spikes in the bladder were accelerated by acetylcholine or ATP and slowed by noradrenaline or isoprenaline.

5 The intrinsic electrical activity and pharmacological properties of the urethra therefore differ from those of the bladder. This may account for the different responses of the two regions in normal function.

Introduction

The bladder and urethra are innervated by both the hypogastric and pelvic nerves. Although both regions have some spontaneous, myogenic activity, nervous input is important in the normal functions of micturition and urine retention which alternate with each other. Despite this, the role of the nerves and the nature of their transmitters are poorly understood.

In the case of the bladder, contraction produced in response to pelvic nerve stimulation is only partially blocked by atropine and is unaffected by phentolamine (Ursillo & Clark, 1956; Ambache & Zar, 1970). The response to hypogastric nerve stimulation is also relatively insensitive to these drugs and close arterial injection of catecholamines produces only a small rise in the bladder pressure in most species (Dave & Dhattiwala, 1976; Creed, 1979). It has therefore been postulated that both these nerves contain non-adrenergic, non-cholinergic fibres and this has led to the suggestion that there may be 'purinergic' nerves to the bladder (Burnstock, Dumsday & Smythe, 1972; Dean & Downie, 1978).

Fewer experiments have been described on the innervation or pharmacology of the urethra. The adrenergic innervation to the urethra is denser than that to the bladder (Gosling & Dixon, 1975) and stimulation of hypogastric nerves causes a powerful contraction. However, experiments on the dog have suggested that non-adrenergic, non-cholinergic fibres also occur in the pelvic and hypogastric nerves to the urethra (Creed & Tulloch, 1978; Creed, 1979). In most experiments on the urethra, drugs have been injected into anaesthetized animals while recording pressure or tension changes (Edvardsen & Setekleiv, 1968; Creed, 1979). In such experiments it is not always possible to distinguish direct effects on the urethra from those produced, for instance, by the opening of the bladder neck or by changes in the cardiovascular system. To avoid these indirect actions the present experiments were carried out on isolated strips of urethra. The actions of several possible transmitter agents on the electrical and mechanical properties were investigated and compared with the effects on strips of bladder.

Methods

Female guinea-pigs (weighing 400 to 800 g) were killed by a blow on the head. The bladder and urethra

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were taken out and dissected free of superficial fat and connective tissue and the mucous layer was removed. To obtain longitudinal strips for mechanical recording, the urethra was divided into two equal parts by mid-dorsal and mid-ventral cuts. Circular strips were taken from the upper and middle thirds of the urethra after opening it with a mid-dorsal cut. The responses to drugs were similar in the longitudinal and circular strips. Longitudinal strips were therefore used for most experiments and the results confirmed on circular strips. Strips of bladder, 2–3 cm long, were cut from the dorsal wall between the ureter openings and the dome of the bladder (i.e. detrusor muscle). The strips were mounted in 10 ml organ baths containing modified Krebs solution at 36°C and gassed with 5% CO_2 in O_2. Urethral strips (1 cm long) were initially stretched to a tension of 300 mg and bladder strips to 1.5 g. A slight reduction in these values occurred as the tension was taken up by elastic elements. Tension was measured with Grass force transducers (F.T.03) and displayed on a grass polygraph. Agonist drugs were added directly to the bath and antagonists were included in the Krebs solution at the required concentrations. Doses quoted are the final concentration in the organ bath. The tissues were in contact with the antagonist drugs for at least 15 min before retesting the action of the agonist drugs.

For electrical recording, mid-dorsal strips, continuous from the dome of the bladder to the urethra, were used. They were mounted in a 3 ml bath with the serosal side uppermost. Modified Krebs solution, which flowed continuously through the bath at 34–36°C, was made hypertonic by addition of 15 g sucrose to 100 ml solution to abolish movement and inactivate nerves. The electrical properties of the smooth muscle cells were recorded with glass microelectrodes filled with 3 M KCl. Drugs were added to the bath and are expressed as the final concentration in the bath. The local concentration before mixing would be 50–100 × greater than this.

Drugs used were acetylcholine chloride (Hopkins and Wilkins), (−)-noradrenaline bitartrate (Sigma), adenosine triphosphate disodium salt (Sigma), 5-hydroxytryptamine (Sigma), (±)-isoprenaline sulphate (Sigma), (−)-phenylephrine hydrochloride (Sigma), atropine sulphate (Sigma), hexamethonium bromide (Sigma), phentolamine mesylate (Ciba), (±)-propranolol hydrochloride (ICI), quinidine gluconate (Drug Houses of Australia), methysergide hydrogenmaleinate (Sandoz), picrotoxin (Sigma). The modified Krebs solution had the following composition (mM): NaCl 120, KCl 5.0 $CaCl_2$ 2.5, $NaHCO_3$ 25.0, $MgSO_4$ 1.0, NaH_2PO_4 1.0 and glucose 11.0; equilibrated with 5% CO_2 in O_2. The drugs were dissolved in Krebs solution before addition to the baths.

Results

Mechanical activity

In the urethral strips spontaneous activity was rarely seen but occasionally slow rhythmic contractions were observed once every 2–3 min. In the bladder spontaneous contractions were common. These occurred at frequencies of 2–4 per min and were up to 5 g in amplitude. The frequency and amplitude were transiently increased by stretch.

Acetylcholine Acetylcholine produced contractions of both urethra and bladder. The threshold was 5×10^{-6} M, and at low doses rhythmic contractions were initiated at the same frequency as those occurring spontaneously. Maximum tensions of 50–300 mg for urethra ($n = 6$) and 8–14 g for bladder ($n = 8$) occurred at 5×10^{-2} M. Contraction of the urethra was slow, taking up to 1 min to reach maximum compared with 0.1 min for bladder. All contractions were maintained. The responses were mimicked by carbachol and antagonized by atropine (7×10^{-7} M) which shifted the dose-response curves to the right. (For urethra ED_{50} increased from 3×10^{-4} M to 1×10^{-2} M). Hexamethonium (3×10^{-5} M) did not affect the responses to acetylcholine.

Biogenic amines Noradrenaline contracted both longitudinal and circular urethral strips but was without action on the bladder. The threshold for the urethra was about 5×10^{-8} M and maximum contractions of 400 mg occurred at 4×10^{-4} M ($n = 10$). The contraction developed rapidly and was maintained for long periods. The response was antagonized by phentolamine (1.3×10^{-6} M), which produced a parallel shift in the dose-response curve (ED_{50} increased from 6×10^{-6} M to 1×10^{-4} M); it was unaffected by propranolol (2×10^{-6} M). The urethra also contracted in the presence of phenylephrine (ED_{50} was about 2×10^{-6} M).

Isoprenaline up to 2×10^{-4} M had no effect on the urethra ($n = 6$) in either the normal relaxed state or when the activity was increased with carbachol (1×10^{-5} M). At higher concentrations isoprenaline produced a small dose-related increase in tension which was abolished by phentolamine, suggesting an action on α-adrenoceptors. Isoprenaline had an inhibitory action on the bladder. At concentrations greater than 1×10^{-7} M it decreased the amplitude of rhythmic contractions and reduced the tension produced by carbachol. The inhibition was antagonized by propranolol. This suggests that the urethra of the guinea-pig has only excitatory α-adrenoceptors whereas the bladder has inhibitory β-adrenoceptors.

5-Hydroxytryptamine (5-HT) caused contraction

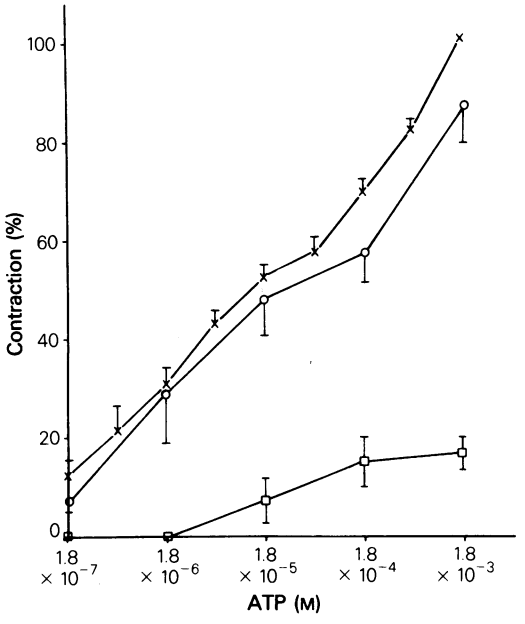

Figure 1 The dose-responses curves of guinea-pig bladder to ATP in the absence (×) and in the presence of two concentrations of quinidine (9×10^{-8} M (○) and 3×10^{-7} M (□)) ($n = 6$). Although the response was still not maximum at ATP 1.8×10^{-3} M, this response was taken as the 100%. The lower dose of quinidine produced a slight reduction in responses but this was not significant ($P > 0.1$ from Student's t test at each concentration of ATP). The bars represent s.e.mean.

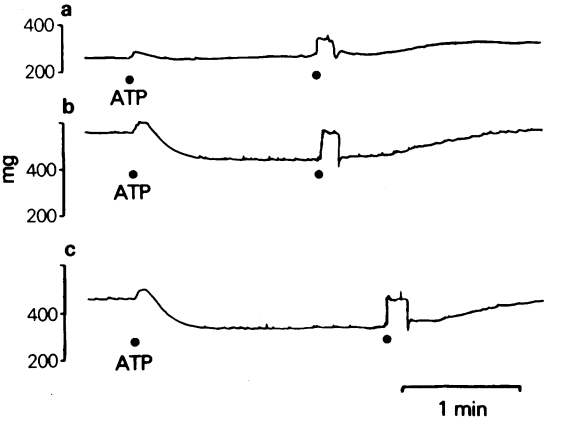

Figure 2 The responses of guinea-pig urethra to ATP (6×10^{-5} M) in the absence of tone (a), when the tone was raised with noradrenaline 6×10^{-5} M (b) and in the presence of noradrenaline and quinidine 1×10^{-6} M (c). The relaxation produced by ATP was not blocked by quinidine. ATP was washed out at the second dot.

of strips of both urethra and bladder. Maximum contractions were produced by 5-HT at 3×10^{-4} M and were about half those produced by acetylcholine (up to 180 mg for urethra ($n = 12$) and 6 g for bladder ($n = 12$)). The initial contraction of the bladder was rapid but the tension was not maintained so that it returned to the resting level in 0.5–2 min. However, the contraction of the urethra was well maintained after a slow initial rise. Methysergide, which directly antagonizes actions on smooth muscle, failed to block responses of the bladder at concentrations up to 1×10^{-6} M. In contrast, the urethral responses to 5-HT were completely blocked by this concentration of methysergide and were reduced by 1×10^{-8} M, 1,000 times more agonist being required for comparable contraction. The bladder response was not blocked by phentolamine (1.3×10^{-6} M) and was therefore not due to stimulation of α-adrenoceptors. Picrotoxin, which blocks transmission at some ganglia (Saum & Groat, 1973), failed to reduce the responses of either bladder or urethra at concentrations up to 8×10^{-6} M.

Adenosine triphosphate The bladder was contracted by ATP with a threshold of 2×10^{-8} M. At higher doses the contraction was larger (Figure 1) and had not reached a maximum at 1×10^{-2} M when the tension was 4–8 g ($n = 10$). ATP at 1×10^{-2} M had no effect on the normal relaxed urethral strips. However, if the tone was raised with noradrenaline (6×10^{-6} M) or carbachol (1×10^{-5} M), ATP had an inhibitory action, the induced tone being reduced to about 50% by 2×10^{-4} M ATP. Compared with the rapid bladder contraction, the development of the inhibition in the urethra was slow (0.5 min to reach a maximum value) but was maintained in the presence of the drug (Figure 2).

Quinidine has been reported to be a specific antagonist of ATP on the bladder and elsewhere (Burnstock *et al.*, 1972). At 9×10^{-8} M it produced only a small and non-significant decrease in the bladder response to ATP (Figure 1). At 3×10^{-7} M there was a greater reduction but the responses to acetylcholine (Figure 3) and 5-hydroxytryptamine were also reduced indicating that the block was not specific. The inhibitory response of ATP on the urethra was not blocked at 1×10^{-6} M but quinidine itself tended to reduce the tone (Figure 2). Atropine (7×10^{-7} M) had no effect on ATP-induced bladder contraction.

Electrical activity

Spontaneous activity In most preparations set up for more than 1 h, all parts of the lower urinary tract had spontaneous activity but the pattern varied with the

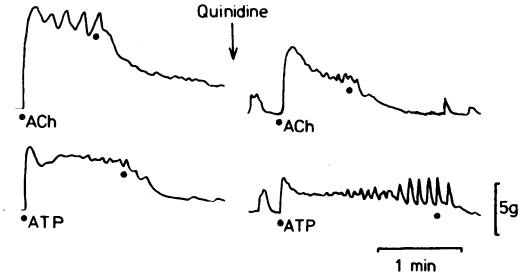

Figure 3 The responses of the bladder to acetylcholine (ACh) 3×10^{-5} M and to ATP 1.8×10^{-5} M before and after addition of quinidine 3×10^{-7} M. Both responses were reduced. The agonist drugs were washed out at the second dots.

site of recording. In cells from the dome of the bladder to within 0–5 mm of the ureter openings (detrusor muscle) the predominant activity was regular spikes at 6–30/min with overshoots of up to 20 mV and a mean (±s.d.) resting potential of 36.3 ± 4.3 mV ($n = 21$) (Figure 4a). Between the ureter opening, cells were found with bursts of 7–10 spikes superimposed on the regular pattern (Figure 4b). Similar activity was seen if strips were cut transversely between the openings. More caudally the bursts tended to be longer and were often separated by periods of intermittent electrical activity (Figure 4c). In the urethra, where cells were relatively difficult to impale satisfactorily due to connective tissue, the only activity seen was infrequent bursts of spikes (once every 1–7 min) separated by quiescent periods. There were 20–30 spikes within most bursts

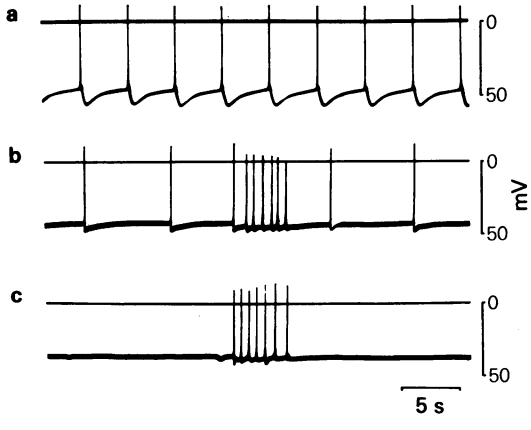

Figure 4 Spontaneous electrical activity recorded from the lower urinary tract of the guinea-pig with microelectrodes. Regular spike activity (a) was seen in the detrusor muscle of the bladder. Between the ureter openings bursts of spikes were superimposed on this pattern (b) and more caudally only bursts occurred (c). In each record the upper trace is zero potential and the lower trace is the membrane potential.

which lasted for 7–10 s. The mean resting membrane potential in the quiescent periods was 42.2 ± 4.0 mV ($n = 26$). If an electrode was withdrawn from one cell and inserted into another less than 1 mm away, it was frequently found that the patterns of activity were out of phase. Furthermore, it was not possible to record spikes evoked by extracellular current pulses suggesting that activity may not spread readily within the urethra. On the other hand, spikes evoked by extracellular electrodes were propagated within the detrusor muscle and into the trigone.

Responses to drugs The response to drugs varied with the pattern of activity. It was confirmed that acetylcholine (5×10^{-6} M) increased the frequency of spikes in the detrusor where the usual activity was regular spiking. It also initiated bursts in the bladder base and urethra. They tended to be shorter (5–8 s) than spontaneous bursts and contain fewer spikes (15–20) but were still separated by quiescent periods.

Noradrenaline (3×10^{-6} M), which inhibited or abolished regular spike activity in the detrusor, was mimicked by isoprenaline whereas phenylephrine had no effect. Conversely, bursts of activity in the bladder base could be initiated by noradrenaline or phenylephrine but not by isoprenaline. After application of the drugs, bursts occurred more frequently and there was a tendency for occasional single spikes or irregular depolarizations between bursts. In the trigone, where many patterns of activity occurred, and also in the urethra, some cells failed to respond to noradrenaline or responses were small and variable. Usually there was no effect on adding 5-HT (1×10^{-6} M) but occasionally a small increase in burst activity was seen in the bladder base.

ATP (2×10^{-6} M) was found to increase the spike frequency in the detrusor without depolarization (Figure 5). The acceleration was transient and could not usually be repeated by a second application. ATP either abolished or did not affect spontaneous bursts. In those cells where bursts were abolished, the pattern of activity often changed during recovery. The bursts occurred less frequently and were preceded by a series of regular spikes before reverting to the original pattern after several minutes (Figure 6).

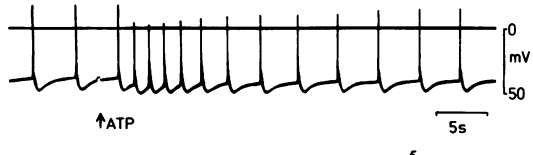

Figure 5 The effect of ATP (1.8×10^{-5} M) on the electrical activity of the detrusor muscle. There was a transient increase in the frequency of action potentials but no general depolarization.

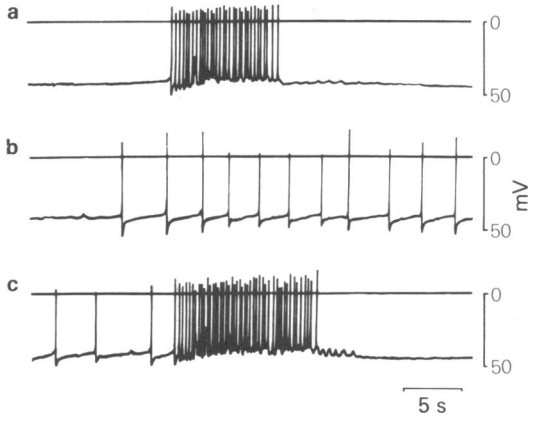

Figure 6 The response of the urethra to ATP (1.8×10^{-5} M). After the burst shown (a), ATP inhibited all spikes for 4.7 min. Activity returned in the form of regular spikes (b) which eventually led to a burst (c). This pattern was repeated several times until isolation bursts again occurred at about every 1.6 min.

Discussion

Smooth muscle cells from all parts of the lower urinary tract showed some spontaneous activity. This ranged from the regular spike activity already described in the detrusor muscle (Creed, 1971a) to infrequent bursts in the urethra. There was no sudden transition from one pattern to another and in the trigone, in particular, there was considerable variation between cells and animals. Bundles of smooth muscle cells extend into this region from the detrusor muscle where the orientation of the cells is predominantly longitudinal. Other bundles pass between the ureter openings or from them to the urethra. Some cells would therefore be expected to have intermediate properties. There is also some evidence that the pattern of activity may be modified by hormones (Creed, 1980).

Recordings from bladder strips made with the double sucrose gap method indicated that spikes are the electrical basis of contraction in this tissue (Kurihara, Kuriyama & Magaribuchi, 1974). In the present experiments electrical and mechanical activity was recorded in different preparations. However, the results suggest that the spike is associated with contraction in the urethra also since there was a parallel effect of drugs on spike activity and contraction. The bursts of activity in the trigone are probably responsible for the rhythmic contractions seen in some of the bladder strips. In the urethra there is much connective tissue so that the smooth muscle cells are dispersed and there appeared to be limited spread of activity. This would explain the asynchronous nature of the spontaneous activity which would not produce a general contraction that could be detected in these experiments.

Acetylcholine stimulated spike activity in the smooth muscle cells of both the bladder and the urethra and produced contraction that was antagonized by atropine but unaffected by hexamethonium. The acceleration of spike activity in the bladder has already been shown to be completely blocked by atropine (Creed, 1971b). These observations suggest that the action of acetylcholine was directly on the smooth muscle cells and that ganglion cells are unlikely to be present in the preparations. That direct action does occur is supported by the finding that in this and other species, cholinergic fibres are closely associated with smooth muscle cells of the urethra and bladder (Gosling & Dixon, 1975).

Noradrenaline produced bladder relaxation, with a decrease in spike activity of the detrusor muscle, and urethral contraction, with initiation of bursts of spikes. The observations support the conclusion that the urethra of the guinea-pig contains only excitatory α-adrenoceptors (Persson & Andersson, 1976) and that inhibitory β-adrenoceptors occur in the bladder (Dave & Dhattiwala, 1976). Histological studies on the guinea-pig have indicated that there is a dense adrenergic innervation of the urethra compared with a sparse bladder innervation (Gosling & Dixon, 1975). The main response to adrenergic nerve activity may therefore be closure of the urethra which aids urine retention.

Another postulated transmitter, ATP, inhibited the urethra but caused excitation of the bladder and could therefore be involved in micturition. In the dog the response of the urethra to close arterial injection of ATP was variable but suggested that the urethral pressure was reduced unless the bladder contraction was sufficiently strong to open the bladder neck so that the urethral and bladder pressures became equal (Creed & Tulloch, 1978). Neither excitatory nor inhibitory actions of ATP were selectively blocked by quinidine as previously recorded for the contractile response of the bladder (Burnstock *et al.*, 1972) or inhibitory responses of the intestine (Burnstock, Campbell, Satchell & Smythe, 1970). Ambache, Killick & Woodley (1977) concluded that ATP was not involved in transmission in the atropinized guinea-pig bladder since its effects were transient and the contractions produced by large doses (1.5×10^{-2} M) were still smaller than responses to nerve stimulation. In the present experiments the response to this dose was also relatively small and the shape of the dose-response curve does not suggest a normal agonist-receptor reaction. It is possible that adenosine, or other purine compounds formed in the bath from ATP, produced relaxation of the urethra and contraction of the bladder. However, detrusor strips

from guinea-pig and rabbit were relatively insensitive to other purines and adenosine caused relaxation (Burnstock *et al.*, 1972; Dean & Downie, 1978). Other purines were not tested on the urethra in the present experiments. Whether ATP is important in transmission in the lower urinary tract cannot yet be determined.

5-HT caused contraction of both the bladder and urethra but the two areas differed in that only the urethral response was blocked by methysergide. The results indicate that tryptaminergic receptors occur on the urethral muscle but may be relatively few or absent on the bladder. This would explain the inability of 5-HT to alter the spike activity in the bladder. However, there was an unexplained difference from results of Ambache & Zar (1970) who found that methysergide reduced contractile responses to 5-HT in strips of guinea-pig bladder.

The urethra therefore resembled the bladder in that contraction was produced in response to acetylcholine and 5-HT. It differed in having no inhibitory β-adrenoceptors, in that ATP caused relaxation and not contraction, and in the response to 5-HT which was completely blocked by methysergide. The two regions also had different intrinsic electrical activity. The differences in transmitter physiology as well as in nerve distribution could account for the characteristic responses of bladder and urethra during normal function.

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