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A COMPARISON OF SPONTANEOUS AND NERVE-MEDIATED ACTIVITY IN BLADDER MUSCLE FROM MAN, PIG AND RABBIT

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SUMMARY

1. Spontaneous activity in bladder muscle strips from man, pig and rabbit has been compared using an *in vitro* superfusion technique. Field stimulation was used to study nerve-mediated activity.

2. Bladder muscle strips from all areas of the rabbit bladder displayed rhythmic spontaneous activity. Spontaneous activity was regularly present in strips from the trigone region in man and pig, but was present in only 18 and 19% respectively of strips from the dome of the bladder.

3. Strength–duration curves in the presence of tetrodotoxin (10^{-7} g/ml) were constructed. The 'chronaxie' of the muscle was found to be considerably shorter than that of other smooth muscles, ranging from 6·1 ms in the rabbit to 12·9 ms in man.

4. Frequency–response curves were constructed using trains of stimuli. The responses were not antagonized by hexamethonium (10^{-4} M), but were markedly inhibited by tetrodotoxin (10^{-7} g/ml), indicating that the responses were mediated by excitation of post-ganglionic nerves.

5. Physostigmine (10^{-7}–5×10^{-6} M) produced a dose-related increase in the contractile response to field stimulation in all three species. Atropine (10^{-8}–10^{-6} M) produced an inhibition of the contractile response, but the maximum degree of inhibition differed considerably between the species. In the rabbit, 58% of the control response was attained, whilst in the pig this was only 22%. Atropine completely abolished nerve-mediated contractions in human bladder muscle.

6. Phentolmaine (10^{-7}–$2{\cdot}5 \times 10^{-7}$ M) had no significant effect on the frequency–response curve in any of the three species, and did not depress the atropine-resistant component in rabbit and pig.

7. It is concluded that nerve-mediated activity in human bladder muscle is exclusively cholinergic, in contrast to most other mammals studied in which there is a significant non-cholinergic component. The finding of a shorter chronaxie in bladder muscle than in other smooth muscles suggests important differences in its physiological properties that merit further investigation.

INTRODUCTION

The majority of studies of bladder smooth muscle have been carried out on laboratory animals, and these studies have revealed important species differences in the nature of the excitatory innervation of the bladder muscle, particularly with

regard to the relative contributions of cholinergic and non-cholinergic mechanisms (Ambache & Zar, 1970; Downie & Dean, 1977; Krell, McCoy & Ridley, 1981).

Several investigators have looked at the responses of human bladder muscle strips to a variety of pharmacological agents added to the organ bath (Todd & Mack, 1969; Bultitude, Hills & Shuttleworth, 1976; Abrams & Feneley, 1976), but such studies do not give any indication of the possible role of these agents as excitatory neurotransmitters. Hindmarsh, Idowu, Yeates & Zar (1977) reported that electrically induced contractions in strips of human bladder, obtained from patients undergoing bladder surgery, were only partially antagonized by atropine, and suggested that acetylcholine was not the sole motor transmitter in the human bladder. More recently, a considerable variation in the degree of atropine sensitivity was reported in muscle strips obtained at operation from different patients, ranging from 35 to 100% inhibition (Sjögren, Andersson, Husted, Mattiasson & Moller-Madsen, 1982; Nergårdh & Kinn, 1983).

All of these studies have used bladder muscle strips obtained at operation from patients undergoing surgery for disorders of the lower urinary tract, and the variation reported in atropine sensitivity may reflect abnormalities in neuromuscular transmission as the result of disease. This study has therefore further examined the nature of nerve-mediated contractions in the human bladder using field stimulation of *in vitro* muscle strips, and for the first time includes muscle obtained at cadaver donor nephrectomy from patients with no known urinary tract disorder. Such information about normal bladder muscle physiology is part of a larger study on the changes found in disordered bladder function.

The pig has proved to be a suitable model for studying clinical disorders of the upper urinary tract (Hodson, Maling, McManamon & Lewis, 1975; Ransley & Risdon, 1978), and there is evidence that the physiology of the pig bladder closely resembles that of man (Melick, Naryka & Schmidt, 1961). As a preliminary investigation of the suitability of the pig as an experimental model for studies on the effects of bladder outflow obstruction on the bladder (a common surgical problem in man), the properties of the pig detrusor have also been studied and are compared here with the findings in man.

Because of the known inter-species variation, and the possibility of variation due to the experimental techniques used, the responses to field stimulation in the rabbit bladder were also examined, since this species has been studied frequently in the past. This helped to provide a basis for comparing the results obtained in bladder muscle from man and pig with the results obtained by previous workers in other species.

In addition to the studies on nerve-mediated activity, differences were found between these species in the patterns of spontaneous activity and in the excitability of the muscle strips to single electrical stimuli as assessed by constructing strength–duration curves. These differences are therefore also reported.

METHODS

Collection of specimens

Human bladder muscle was obtained from two sources, either at cadaver donor nephrectomy, following brain death as a result of road traffic accident or intracerebral haemorrhage, or at operation from consenting patients undergoing surgery for lower urinary tract disorders. The study

was approved by the hospital ethical committee. Bladder muscle obtained from fifty-nine patients has so far been evaluated (sixteen obtained at cadaver donor nephrectomy and forty-three at operative surgery).

In the case of cadaver donor nephrectomy specimens, a strip of bladder muscle was taken from the dome of the bladder within a few minutes of cessation of the circulation. The majority of these patients had received the α-blocking agents phenoxybenzamine and phentolamine immediately prior to nephrectomy to improve the renal blood flow, but no anticholinergic drugs (e.g. atropine) or anaesthetic agents were administered. The specimen was transported to the laboratory in Krebs solution cooled to 5–10 °C; operative specimens of human bladder and specimens from pig bladders were transported in a similar fashion.

Operative samples included specimens from patients undergoing transvesical prostatectomy for benign prostatic enlargement. In these patients, a strip of muscle was taken from the anterior wall of the dome of the bladder in making the incision into the bladder. In order to obtain specimens from the trigone of the bladder to study spontaneous activity, trigonal strips were taken from the operative specimen in a small group of patients following total cystectomy for bladder carcinoma. None of these patients undergoing operative surgery received anticholinergic drugs (e.g. atropine), either in pre-medication or during surgery prior to biopsy; in addition none of them received either α- or β-adrenergic blocking agents.

Specimens of pig bladder were also obtained from two sources. Specimens from the anterior wall of the dome of the bladder were obtained at operation under 2% halothane anaesthesia from Landrace boars which were acting as controls in a study of the effects of bladder outflow obstruction on detrusor muscle physiology. This source was supplemented by bladder muscle obtained from male pigs freshly killed in the abbattoir by exsanguination, particularly to obtain trigonal muscle; the bladder was rapidly excised during evisceration of the animals, and then opened and drained of urine.

Rabbit bladders were obtained from New Zealand white male rabbits weighing approximately 2 kg. These were stunned by a blow to the back of the head and exsanguinated. The bladder was excised and placed in Krebs solution at room temperature, and strips were cut from the anterior dome and the posterior wall of the bladder.

Muscle strip studies

From each type of bladder specimen, strips of muscle measuring approximately 7 mm by 1·5 mm unstretched were prepared, using an operating microscope to enable strips with good longitudinal alignment of the muscle bundles to be dissected. Fine silk ligatures were tied to each end of the strip which was then mounted in a specially constructed Perspex organ chamber with a capacity of 0·2 ml and continuously perfused with Krebs solution between 34·5 and 36·5 °C in a superfusion apparatus (Brading & Sibley, 1983). Within the organ chamber the muscle strip was mounted between platinum ring electrodes 1 cm apart. The Krebs solution had the following composition (mM): Na, 136·9; K, 5·9; Ca, 2·5; Mg, 1·2; Cl, 133·6; HCO_3, 15·5; H_2PO_4, 1·2; glucose, 11·5; bubbled with 97% O_2, 3% CO_2, pH 7·4 at 36 °C.

The strips were allowed to equilibrate for at least 1 h, during which a resting tension of 0·5 g was applied. Isometric tension changes were measured using Pioden (U.K.) UF 1 tension transducers, and after amplification the contractions were recorded on a Watanable (Japan) multi-channel pen recorder. Using six organ baths, six tissues could be studied simultaneously; this is particularly useful when monitoring the effects of agonists and antagonists on electrically evoked contractions, since time-dependent changes can be monitored simultaneously in control strips.

The electrical impulses for field stimulation were delivered from a Grass (U.S.A.) S48 stimulator. Tetrodotoxin (TTX, 10^{-7} g/ml) was used to distinguish between nerve-mediated contractile responses and those due to direct muscle stimulation. Details of the stimulation parameters used are given in the appropriate sections in the Results.

At the commencement of each experiment, a control response to a 10 s application of 5×10^{-5} M-carbachol was established (this dose produces a near maximal contraction) so that subsequent responses could be compared with this control if desired.

The drugs used were: carbamylcholine chloride (carbachol), atropine sulphate (BDH Chemicals Ltd.), phentolamine mesylate (Ciba Laboratories Ltd.), physostigmine sulphate (Burroughs Wellcome), hexamethonium bromide and tetrodotoxin (Sigma Chemicals).

Statistics

When testing the effects of agonists or antagonists on strength–duration curves and frequency–response curves, each strip served as its own control, though additional untreated strips were also studied to monitor any time-dependent changes. Student's t test was used to compare differences in responses between the control and experimental curves. Data were regarded as significant at the $P < 0.05$ level.

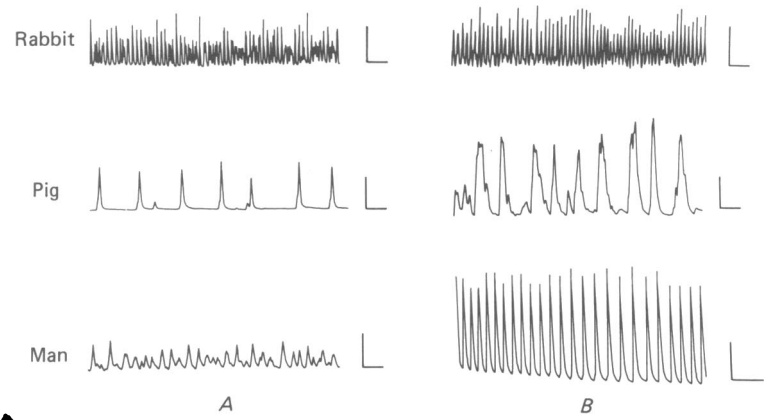

Fig. 1. Spontaneous activity in detrusor muscle strips from rabbit, pig and man. *A*, strips taken from the anterior dome. *B*, strips taken from the trigone in man and pig, and the posterior wall of the rabbit bladder. Vertical bars represent 0·25 g of tension. Horizontal bars represent 1 min.

RESULTS

Spontaneous activity (Fig. 1)

Rhythmic spontaneous activity was present in all strips taken from the rabbit bladder, with an average frequency of 7·3 contractions/min (n, number of muscle strips = 30).

In contrast, spontaneous activity was not regularly present in strips from the dome of the pig bladder. Only 19% of 191 strips showed a degree of spontaneous activity. On the other hand spontaneous activity was seen in the majority of strips taken from the trigone region, being present in seventeen out of nineteen strips, or 89%. Unlike the rapid and frequent contractions seen in rabbit detrusor strips, those occurring in the pig were less frequent, averaging 1·1 contractions/min.

The human bladder showed a similar pattern to the pig bladder. 20% of 139 strips taken from the dome of control bladders showed spontaneous activity, and when dome strips from all human sources were considered this figure was only 18%. The only source of trigonal muscle from human bladders was provided by the cystectomy specimens, and five out of seven strips (71%) showed spontaneous activity. The frequency of spontaneous activity was again less than in the rabbit, averaging 2·2 contractions/min.

During the course of experiments involving electrical field stimulation, the spontaneous activity in muscle strips from human and pig bladder tended to decrease. In the rabbit, spontaneous activity persisted during stimulation experiments, and

this activity was not diminished by the addition of 10^{-4} M-hexamethonium, 5×10^{-7} M-atropine, or TTX (10^{-7} g/ml); in some strips a slight increase in amplitude of the contractions was seen in the presence of atropine and TTX.

Strength–duration curves

Single electrical stimuli were used to construct strength–duration curves for each species, and then repeated in the presence of either 5×10^{-7} M-atropine (a dose

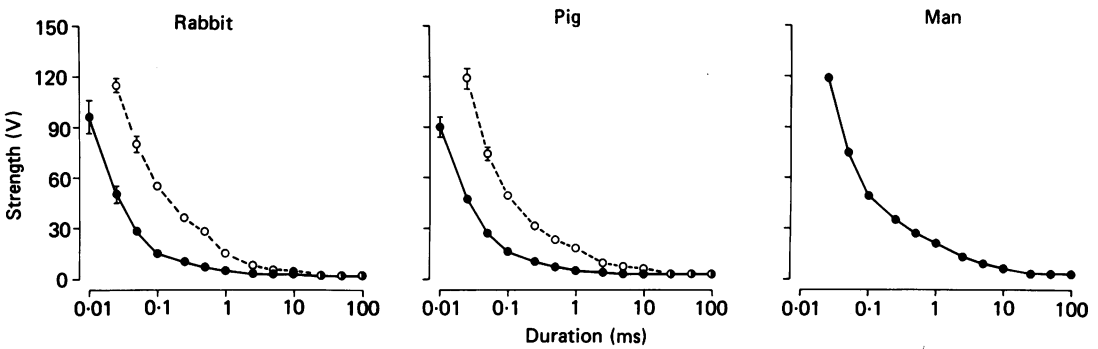

Fig. 2. Strength–duration curves for threshold mechanical responses to single electrical impulses in detrusor muscle strips from rabbit bladder ($n = 8$, three animals), pig bladder ($n = 16$, seven animals), and human bladder ($n = 14$, six patients). ●, control responses. ○, responses in rabbit and pig after a 15 min exposure to TTX, 10^{-7} g/ml; in human bladder, the threshold responses were not significantly altered in TTX. Vertical bars indicate S.E. of the means.

sufficient to abolish the contractile response to 5×10^{-5} M-carbachol) or TTX (10^{-7} g/ml). At each stimulus duration the threshold voltage to elicit a contraction was determined. In the rabbit, strength–duration curves were determined in strips cooled to 25–26 °C to reduce the level of spontaneous activity, which otherwise made it difficult to detect threshold contractions. In the pig and human bladders, cooling was not necessary.

From these curves it was possible to estimate the 'chronaxie' of the tissues (the pulse duration which had a threshold intensity twice the minimum intensity to induce a mechanical response), which can be used to compare the excitability of different tissues (Paton, 1955).

The strength–duration curve for rabbit detrusor muscle is illustrated in Fig. 2. In the control curve, the chronaxie was 1·2 ms ($n = 8$). In the presence of atropine, the strength–duration curve was unaltered. However, in the presence of TTX there was a decrease in the sensitivity of the preparation to electrical stimulation, seen as a shift to the right in the strength–duration curve, with the chronaxie increased to 6·1 ms. This difference between the two curves indicates that a nerve-mediated response was involved in the contractile responses in the control curve.

The strength–duration curve for pig detrusor muscle (Fig. 2) showed a similar pattern to that seen in the rabbit, with a chronaxie of 1·4 ms ($n = 16$) in the control curve. Once again, the strength–duration curve was unaltered in the presence of

atropine, whilst TTX caused a significantly decreased sensitivity to electrical stimulation with a shift of the curve to the right and the chronaxie increased to 11·2 ms.

In contrast to the results in the rabbit and the pig, the strength–duration curve obtained for human detrusor muscle in the presence of TTX did not differ significantly from the control curve, indicating that the control curve resulted from direct muscle stimulation (Fig. 2). The chronaxie of the tissues was 11·9 ms in the control curve and 12·9 ms in the presence of TTX ($n = 14$). These values are similar to those seen for direct muscle stimulation in the pig and the rabbit, suggesting that in human detrusor muscle a single electrical impulse is incapable of releasing sufficient transmitter from the intramural nerve endings to evoke a response.

Nerve-mediated responses of the bladder

Choice of stimulation parameters. Nerve-mediated responses of the bladder were studied using electrical impulses of short duration to selectively stimulate the intramural nerves. In order to choose the optimum parameters for producing stimulation of the nerve fibres without causing direct smooth muscle excitation, critical evaluation of the response obtained was carried out by TTX blockade. A dose of 10^{-7} g/ml was used, higher doses being no more effective. As was discovered with the strength–duration curves, stimuli of short duration could cause direct muscle excitation when high voltages were used.

In all three species, a stimulating voltage of 50 V and a stimulus duration of 0·05 ms were found to be satisfactory for eliciting a nearly pure nerve-mediated response. In the rabbit 3 s trains of stimuli were used as the maximum response was achieved in this time, whilst in pig and man a 5 s train was necessary. Successive trains of stimuli were given at least 2 min after the previous contraction had returned to base line. Successive frequency–response curves repeated with a 15 min interval between them were similar to the initial curves, and any time-dependent changes were small (generally less than 10%).

Using these parameters, abolition by TTX was complete at all frequencies in the rabbit (Fig. 4), whilst in the pig it was complete at 15 Hz and below; between 20 and 50 Hz there was a small TTX-resistant contraction, presumably due either to direct muscle excitation or to release of transmitter by direct depolarization of the nerve terminals, but amounting to only 3% at 50 Hz (Fig. 5). In human detrusor muscle strips this TTX-resistant component was more evident, increasing from 0·5% at 10 Hz to 7% at 50 Hz.

Responses to field stimulation were not antagonized in any species by 10^{-4} M-hexamethonium, indicating that the responses were mediated by excitation of post-ganglionic nerve fibres.

Cholinergic contribution to the nerve-mediated response. In each species, physostigmine enhanced the excitatory responses induced by field stimulation; enhancement was seen with 10^{-7} M-physostigmine, and a maximum effect was obtained between 10^{-6} and 5×10^{-6} M. Fig. 3 illustrates the effects of physostigmine on the field stimulation response in human bladder muscle.

Atropine (10^{-8} to 10^{-6} M) caused a dose-dependent inhibition of the response to field stimulation. A maximal degree of inhibition was achieved with a concentration of

5×10^{-7} M-atropine. This dose was therefore used to assess the cholinergic contribution to the field stimulation response.

In the rabbit, atropine only partially inhibits the response to field stimulation (Figs. 4 and 5). Atropine was least effective at 1 Hz, when 86% of the control response persisted ($n = 12$). At higher frequencies the blockade was more effective, but 56–64%

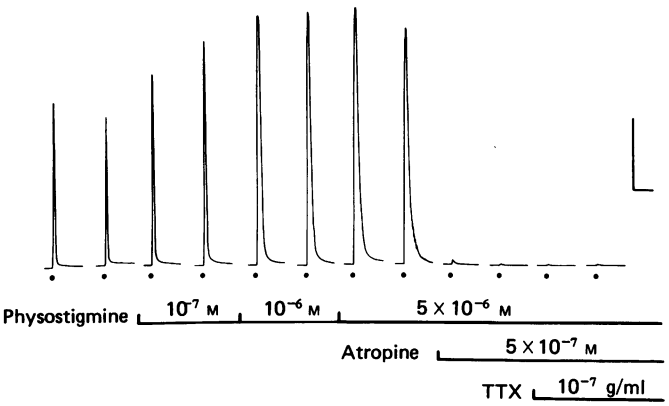

Fig. 3. Field stimulation-induced detrusor contractions at 20 Hz (5 s) in human bladder before and after exposure to increasing concentrations of physostigmine. Stimulus delivered every 7½ min. The effects of adding first atropine and then TTX on the response is shown. Vertical bar represents 2 g of tension. Horizontal bar represents 2 min. Stimulus strength 50 V; stimulus duration 0·05 ms.

of the control response still persisted at 3–40 Hz. This atropine-resistant response was completely abolished by the addition of TTX, and thus represents a large non-cholinergic contribution to the nerve-mediated contractions of rabbit detrusor muscle.

In the pig, atropine was again only partially effective in inhibiting the response to field stimulation, although inhibition was more pronounced than in the rabbit (Figs. 4 and 5). As in the rabbit, atropine was least effective at low frequencies, with 80% of the control response persisting at 1 Hz ($n = 21$). At higher frequencies, however, the contribution of this non-cholinergic transmission declined, with only 17% of the control response present at 50 Hz. TTX abolished the atropine-resistant response below 15 Hz and markedly reduced it at higher frequencies, indicating that the response was nerve mediated.

In contrast to the findings in rabbit and pig, atropine produces an effective blockade of the frequency–response curve at all frequencies in human detrusor muscle (Figs. 4 and 5). The response at 5 Hz is either completely abolished or only just detectable, whilst at other frequencies only a small response persists, from 1% of control at 10 Hz to 7% at 50 Hz ($n = 23$). The addition of TTX does not significantly reduce the response further, indicating that nerve-mediated contractile responses in human detrusor muscle are purely cholinergic. Atropine was as effective in abolishing nerve-mediated contractions in bladder muscle obtained from patients undergoing

prostatectomy (no α-blockers given) as in muscle obtained at cadaver donor nephrectomy (exposed to α-blockade).

In five strips from prostatectomy patients the atropine-resistant response at 50 Hz was 10% or greater (maximum 20%); however, this was accompanied by a similar resistance to blockade by TTX and therefore did not represent a non-cholinergic nerve-mediated response.

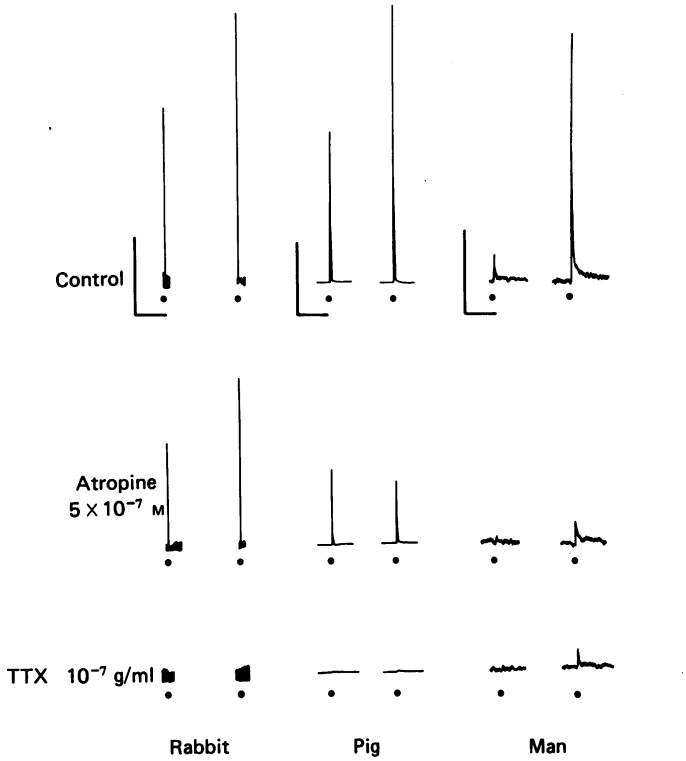

Fig. 4. The effects of atropine and TTX on field stimulation-induced detrusor contractions in rabbit, pig and man. Responses recorded to stimulation at 5 and 20 Hz (first and second contractions respectively). Horizontal bars represent 10 min. Vertical bar represents 0·5 g of tension in human bladder and 1 g of tension in pig and rabbit bladder. Stimulus strength 50 V; stimulus duration 0·05 ms. 3 s train of impulses in the rabbit, 5 s train in pig and man.

In combination with 5×10^{-7} M-atropine, 5×10^{-6} M-physostigmine did not enhance the atropine-resistant response in any of the three species, providing further evidence for its non-cholinergic nature.

Adrenergic contribution to the nerve-mediated response. Histochemical fluorescence studies demonstrate the presence of adrenergic nerve fibres in bladder muscle, although they are sparsely distributed (El-Badawi & Schenk, 1966). β-receptors mediating detrusor relaxation and α-receptors mediating detrusor contraction have been demonstrated in the bladder of many species (Taira, 1972).

The possibility that adrenergic nerves might contribute to detrusor contraction by stimulation of α-receptors was therefore considered in these three species. The effects

of the α-blocker phentolamine on the frequency–response curve of muscle strips from the bladder dome were assessed using concentrations of 10^{-7} and $2{\cdot}5 \times 10^{-7}$ M; higher concentrations may cause non-specific inhibition by virtue of a local anaesthetic action (Bauer, 1982). There was no significant effect on the frequency–response curve at either of these concentrations in any of the three species.

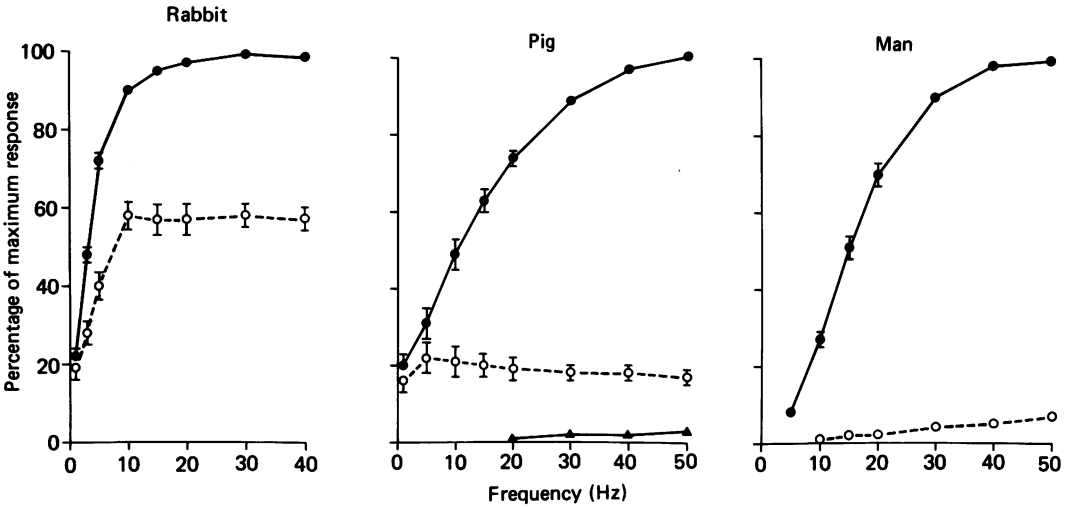

Fig. 5. Frequency–response curves before (●) and after (○) a 15 min exposure to 5×10^{-7} M-atropine. In the pig, the curve obtained in TTX (10^{-7} g/ml) is also shown (▲); in the rabbit, TTX completely abolishes the response, whilst in man TTX does not cause any reduction in the curve obtained in atropine alone. Rabbit, $n = 12$ (four animals); pig, $n = 21$ (eight animals); man, $n = 23$ (ten patients). Vertical bars represent S.E. of the means. Stimulus strength 50 V; stimulus duration 0·05 ms. 3 s train of impulses in the rabbit, 5 s train in pig and man.

Human bladder muscle strips included those obtained at prostatectomy (no previous exposure to α-blockers) since strips obtained from patients undergoing cadaver donor nephrectomy had been exposed to α-blocking agents *in vivo* prior to removal from the bladder.

When a combination of $2{\cdot}5 \times 10^{-7}$ M-phentolamine and 5×10^{-7} M-atropine was used in muscle strips from the rabbit ($n = 4$) and the pig ($n = 6$), a persistent contractile response to field stimulation was seen. This persistent response was similar in magnitude to that seen in the presence of atropine alone in concurrent control strips. Thus, the atropine-resistant response seen in rabbit and pig bladder muscle strips is not due to an α-adrenergically mediated mechanism.

DISCUSSION

The reason for the pronounced differences in spontaneous activity between rabbit detrusor muscle strips on the one hand and pig and human detrusor strips on the other is not clear. The electrical properties of isolated rabbit detrusor muscle strips during spontaneous activity have been studied using the sucrose gap and micro-

electrodes (Anderson, Goellner & Pierce, 1972; Creed, Ishikawa & Ito, 1983). Single or complex action potentials occurred either immediately before or very early during the generation of initial tension of a contractile event. These action potentials were often preceded by a small amount of transmembrane depolarization, suggesting the presence of visceral muscle pace-maker cells in the population.

The control strength–duration curves for rabbit and pig detrusor muscle strips had chronaxie of 1·2 and 1·4 ms respectively. These times are similar to those considered to be characteristic for excitation of post-ganglionic fibres in guinea-pig ileum (0·2 ms: Paton, 1955), human renal calyx (0·6 ms: Longrigg, 1975), and rabbit detrusor muscle (0·38–0·39 ms: Downie & Dean, 1977). However, the chronaxie in the presence of TTX and hence for direct muscle stimulation was 6·1 ms in rabbit detrusor, and 11·2 ms in pig detrusor. Although the chronaxie of smooth muscle from rabbit portal vein was shown to be approximately 12·5 ms (Holman, Kasby, Suthers & Wilson, 1968), these times are otherwise considerably shorter than the estimates for the chronaxie of smooth muscle of intestine (80 ms: Paton, 1955) and ureter (range 30–180 ms: Bozler, 1938; Weiss, Holcomb & Bassett, 1972).

Since TTX is known to block the generation of action potentials in nerve fibres but not in smooth muscle cells (Kuriyama, Osa & Toida, 1966), the shift in the strength–duration curves of pig and rabbit detrusor in the presence of TTX indicates that the control responses to electrical stimulation were nerve-mediated. The inability of atropine to block this nerve-mediated response suggests that a non-cholinergic transmitter is involved in producing this response to a single stimulus. This corresponds well with the major contribution to detrusor contraction made by non-cholinergic mechanisms at low frequencies in the frequency–response curves of these two species. The ability of a single stimulus to cause a contraction suggests a close anatomical innervation or a strong potency of the transmitter involved.

In contrast, in human detrusor muscle, which appears to lack this non-cholinergic innervation, no nerve-mediated control curve is seen. The strength–duration curve is not significantly altered by TTX, indicating that the control curve is the result of direct muscle stimulation, and the chronaxie of 11·9 ms (control curve) and 12·9 ms (in TTX) are similar to those for direct stimulation in the pig detrusor.

When frequency–response curves were constructed in rabbit detrusor muscle, a significant proportion of the contractile response was resistant to blockade by atropine. The atropine resistance was most marked at low frequencies and became less at higher frequencies. This is in agreement with the findings of previous workers in the rabbit (Downie & Dean, 1977), and has also been found in other species such as the guinea-pig (Krell *et al.* 1981). A small atropine-resistant component was also demonstrated in the pig in these experiments; this was again most evident at low frequencies. This suggests two modes of neurotransmission to the detrusor muscle of these species, a non-cholinergic component that is predominantly responsible for contractile responses at low stimulation frequencies, and a cholinergic component that comes into effect at higher frequencies.

Further evidence for two modes of neurotransmission in the rabbit bladder has come from studies on the electrical activity of the detrusor muscle cells during field stimulation (Creed *et al.* 1983). Application of short current pulses produced an initial excitatory junction potential with a superimposed spike, followed by a late depolarization. All of these responses were abolished by TTX. Atropine abolished the

late depolarization, indicating that this was due to activation of the muscarinic receptor. However, atropine had no effect on the early depolarization or the spike, indicating that these events were mediated by a non-cholinergic mechanism.

Since α-adrenergic blocking agents do not inhibit the response to field stimulation in any of the three species and do not depress the atropine-resistant component in the rabbit and pig, it is unlikely that an α-adrenergically mediated mechanism is responsible for this atropine resistance. This is in agreement with the view of Dean & Downie (1978), who also found no effect of α-adrenergic blocking agents on field stimulation responses in the rabbit bladder, suggesting that a non-cholinergic, non-adrenergic transmitter was responsible. Although the identity of this transmitter has not been established, adenosine triphosphate or a related purine compound has been suggested (Burnstock, Cocks, Crowe & Kasakov, 1978; Andersson, Husted & Sjögren, 1980).

Histological studies on human detrusor muscle have shown a rich supply of acetylcholinesterase-positive nerve fibres, whilst noradrenergic nerves are few and found chiefly in association with blood vessels (Gosling, Dixon & Humpherson, 1983). However, the possible existence of non-cholinergic, non-adrenergic nerves and their functional significance is unresolved.

In the studies reported here, the almost complete abolition of the contractile response to field stimulation in human detrusor muscle strips by atropine indicates that cholinergic transmission plays the dominant role in nerve-mediated detrusor contractions, with probably little or no contribution by non-cholinergic mechanisms. The use of detrusor muscle from two different sources, both of which demonstrated this cholinergic dominance, provides convincing evidence for this view. The administration of α-adrenergic blocking agents to those undergoing cadaver donor nephrectomy might have masked an α-adrenergic contribution to detrusor contraction; however, since none of the patients undergoing operative surgery received α-adrenergic blocking agents, this seems unlikely.

It is also interesting to note that little detrusor contraction occurs at stimulation frequencies below 5 Hz in the control curve in human detrusor muscle, since non-cholinergic mechanisms seem to be responsible for contraction at these low frequencies in the rabbit and pig. Thus, the detrusor contraction in man at 5 Hz is only 8% of the maximum response, compared with 31 and 72% respectively in the control curves of the pig and rabbit.

It was found to be important to test whether an apparent atropine-resistant response was nerve-mediated or not. Thus, in five patients undergoing prostatectomy an atropine-resistant response of 10% or greater of the control response persisted at 50 Hz (maximum 20%). However, when the nature of this persistent response was examined with TTX, it was found to be TTX resistant as well. This could be a reflexion of an increased sensitivity of bladder muscle to direct stimulation in certain patients with disordered bladder function, and this is currently being investigated. It may also explain the apparent atropine resistance reported by Sjögren *et al.* (1982) and Nergårdh & Kinn (1983); although TTX was used to test its effects on the field stimulation response in some strips in these studies, it does not appear to have been used in those strips displaying atropine resistance to evaluate the nature of the persistent contraction.

In conclusion, these studies have demonstrated some important differences in the

properties of human detrusor muscle compared with other animal species. In particular, neurotransmission appears to be exclusively cholinergic, with no evidence for a non-cholinergic component. Care must therefore be taken when attempting to extrapolate the results of physiological and pharmacological studies of bladder smooth muscle in animal species to man.

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