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PHARMACOLOGICAL CHARACTERIZATION OF THE EXCITATORY INNERVATION TO THE GUINEA-PIG URINARY BLADDER *in vitro*: EVIDENCE FOR BOTH CHOLINERGIC AND NON-ADRENERGIC-NON-CHOLINERGIC NEUROTRANSMISSION

ROBERT D. KRELL,[1] JEAN L. McCOY & PETER T. RIDLEY

Smith Kline and French Laboratories, Department of Pharmacology I, 1500 Spring Garden Street, P.O. Box 7929 Philadelphia, PA. 19101, U.S.A.

1 Field stimulation of strips of guinea-pig isolated urinary bladder with 5 s trains at 0.1 to 15 Hz resulted in frequency-dependent, reproducible contractions.

2 At concentrations of 1 and 4×10^{-7}M and 1×10^{-6}M, atropine produced a variable, partial inhibition of contractions at all frequencies but was most effective at frequencies of 3 Hz or more.

3 Tetrodotoxin (TTX), 5×10^{-7}M, inhibited contractions at all frequencies by 80 to 90%.

4 Physostigmine, 2×10^{-6}M, significantly enhanced the contractile response to frequencies of less than 10 Hz but did not enhance responses resistant to inhibition by atropine. Hexamethonium, 1×10^{-4}M, slightly enhanced the contractile response to frequencies of 4 Hz or greater.

5 (±)-Propranolol (5×10^{-6}M), guanethidine (1×10^{-6}M), phentolamine (5×10^{-6}M) and clonidine (3×10^{-8}M) each enhanced the contractile response to field stimulation.

6 Contractile responses obtained in the presence of atropine (4×10^{-7}M) and guanethidine (1×10^{-6}M) increased with time and were inhibited 60 to 80% by TTX (5×10^{-7}M).

7 It is concluded that the cholinergic nervous system contributes, in part, to electrically-induced excitatory contractions of the isolated urinary bladder of the guinea-pig. Concomitant sympathetic stimulation appears to serve an inhibitory role. In addition, a major portion of the contractile response appears to be due to a non-cholinergic non-adrenergic, as yet unidentified, substance.

Introduction

Neuronally-induced excitatory responses of the urinary bladder of most mammalian species are inhibited only partially by atropine or other antimuscarinic agents (Langley & Anderson, 1895; Edge, 1955; Ursillo, 1961; Chester & Thorp, 1965; Huković, Rand & Vanov, 1965; Dumsday, 1971; Carpenter, 1977; Downie & Dean, 1977; Johns & Paton, 1977). One explanation for this partial 'atropine-resistance' is that, in addition to acetylcholine, released via cholinergic nerves, a non-adrenergic non-cholinergic (NANC) neurotransmitter also contributes to the excitatory responses to nerve stimulation in the urinary bladder (Ambache & Zar, 1970; Burnstock, Dumsday & Smythe, 1972; Burnstock, Cocks, Crowe & Kasakov, 1978; Dean & Downie, 1978).

Studies in the guinea-pig contrast with those in the urinary bladder of most other species in that atropine has little or no effect on nerve-mediated excitatory responses (Chester & Thorp, 1965; Ambache & Zar, 1970). This suggests that NANC nerves mediate most of the excitatory response in the urinary bladder of this species. In the present experiments, the contribution of both cholinergic and adrenergic nerves to the excitatory response in the guinea-pig urinary bladder have been re-evaluated. Some of these results have been published previously (Krell, McCoy & Ridley, 1980).

Methods

General

Male albino guinea-pigs (400 to 800 g) were killed by a blow to the head. The entire urinary bladder was removed, emptied, and sectioned to form a single strip approximately 5 cm in length. In early studies, the mucosal surface was carefully removed; however, results were not influenced by this procedure and later studies used intact bladder strips. The tissue was

[1]Present address: ICI Americas Inc., Biomedical Research Dept, Wilmington, DE 19897, U.S.A.

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suspended in a 25 ml tissue bath, subjected to a resting load of 1 g and bathed at 37.5°C in modified Krebs solution of the following composition (mM): NaCl 118, KCl 4.6, $CaCl_2$ 1.8, $MgCl_2$ 0.5, $NaHCO_3$ 24.9, NaH_2PO_4 1.0 and glucose 11.1. The bath was constantly bubbled with 95% O_2 and 5% CO_2. Isometric contractions were measured with Grass FTO3C force-displacement transducers and recorded on a Sanborn polygraph. Tissues were allowed a 90 min equilibration period during which there were frequent changes of the bathing fluid.

Frequency-response curves

Field stimulation was accomplished by insulated platinum electrodes positioned above and below the tissue using a square wave stimulator (American Electronic Co.) 0.1 to 15 Hz, supramaximal voltage, 0.5 ms duration, 5 s trains at 1 min intervals. While frequencies above 15 Hz provided a greater contractile response, they were considered outside the normal physiological range for this tissue and were not routinely employed (Milne, Booth & deGroat, 1978).

Two frequency-response curves were obtained from each tissue. After completion of the first (control) curve, tissues were rinsed repeatedly with the modified Krebs solution for 60 min. Following the 60 min recovery period, drugs were added to the tissue bath for 30 min before and during the construction of a second frequency-response curve. Frequency-responses obtained in the presence of drugs were expressed as percentage changes from the control (first) curve. Student's t test was used to compare differences in responses on nontransformed data. Data were regarded as significant at the $P < 0.05$ level.

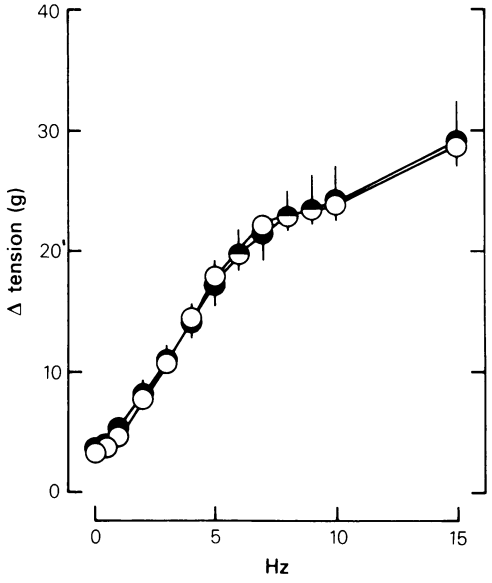

Figure 1 Reproducibility of frequency-response curves to field stimulation in guinea-pig isolated urinary bladder. After a 90 min stabilization period, a frequency-response curve was obtained (●); 90 min later, a second frequency-response curve (○) was obtained in the same tissue. Stimulation parameters were: supramaximal voltage, 0.5 ms duration; 5 s trains at 1 min intervals. Each point is the mean of 7 observations. Vertical lines show s.e.mean; where these are omitted, they are smaller than the symbol for the mean.

Drugs

The following were used: atropine sulphate (ICN-K&K Labs., Plainview, N.Y.); hexamethonium bromide (Sigma Chemical Co., St Louis, MO); tetrodotoxin (Calbiochem Behring Corp., LaJolla, CA); clonidine, (±)-propranolol hydrochloride, guanethidine sulphate and phentolamine hydrochloride (Smith Kline & French, Philadelphia, PA). Except for tetrodotoxin, all compounds were prepared as stock solutions in distilled water and stored at −20°C. Concentrations refer to final bath molar of the base. Tetrodotoxin was prepared in a citric acid buffer and stored at −20°C.

Results

Control frequency-response curves

A substantial intertissue variability in the response to electrical stimulation was observed. For example, in strips prepared from 7 weight-matched guinea-pigs, responses to a 5 s train of pulses delivered at 15 Hz ranged from 18.6–44.0 g. On the basis of these data, it was desirable to evaluate the reproducibility of the frequency-response curve in individual tissues.

Successive frequency-response curves, separated by 90 min, were reproducible (Figure 1). Thus each tissue served as its own control; the effects of drugs were examined on the second frequency-response curve and compared to the control curve obtained 90 min earlier.

Effect of atropine and tetrodotoxin

Atropine, 1×10^{-7}M, partially inhibited bladder contractions at all frequencies (Figure 2a); the inhibition was greatest at frequencies above 3 Hz, while at lower than 3 Hz atropine blockade appeared to vary directly with frequency. Inhibition at from 3 to 15 Hz ranged from 37–55%. Inhibition by atropine appeared to diminish slightly at frequencies of about 7–10 Hz resulting in a 'notching' of the curve.

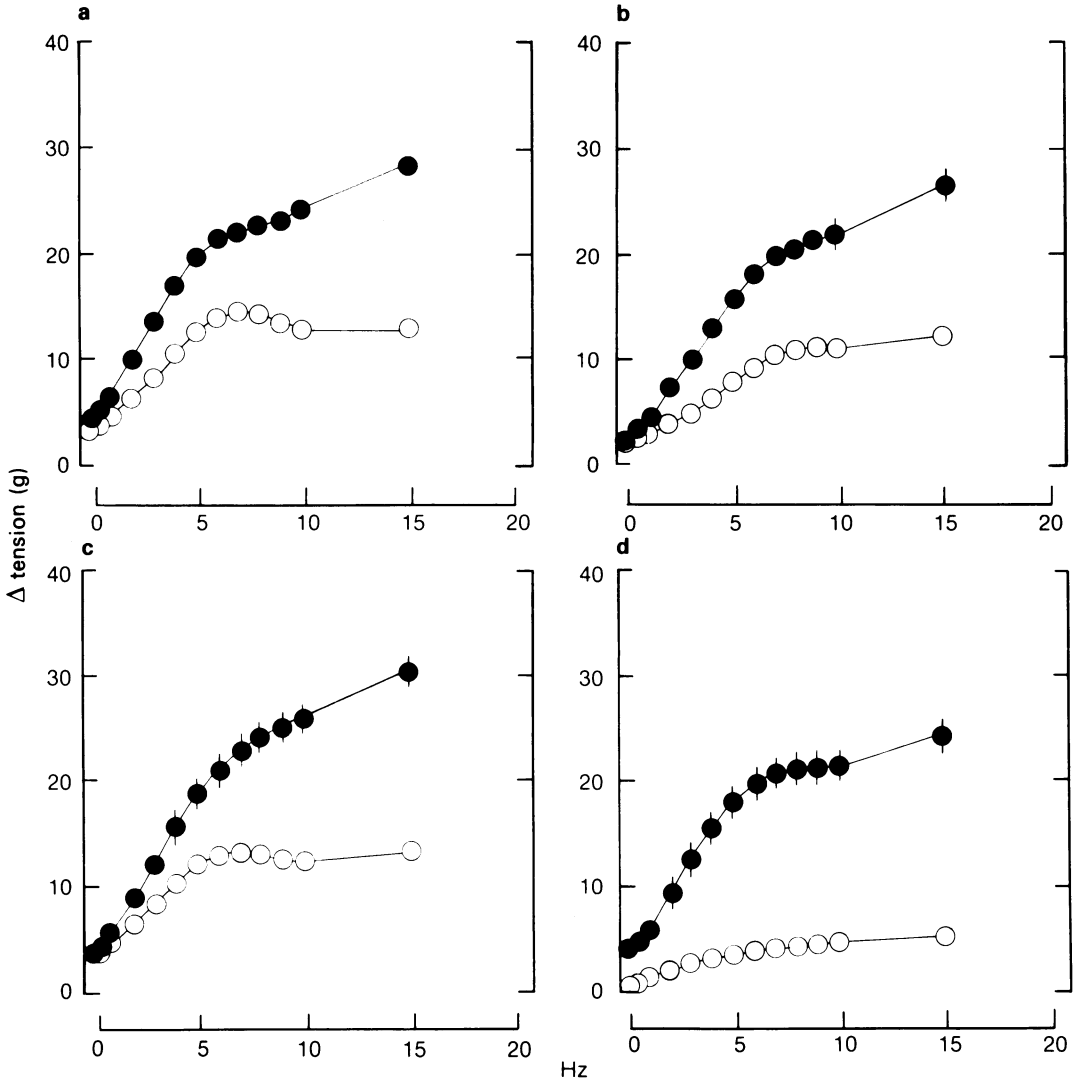

Figure 2 The effect of atropine and tetrodotoxin on electrically-induced excitatory responses in guinea-pig isolated urinary bladder. Sixty min after the first (control) frequency-response curve, tetrodotoxin or one of three concentrations of atropine was added to the bath and, following a 30 min incubation, a second frequency-response curve was obtained in the presence of the agent. Solid symbols (●) are control curves; open symbols (○) represent experimental curves. (a) Atropine 1×10^{-7}M; (b) atropine 4×10^{-7}M; (c) atropine 1×10^{-6}M; (d) tetrodotoxin, 5×10^{-7}M. Stimulation parameters were as described in Figure 1. Inhibition of responses by each concentration of atropine and by tetrodotoxin was significantly ($P < 0.05$) different from controls at all frequencies. There were no significant ($P > 0.05$) differences in the absolute tension increase in response to any frequency of the three control curves for atropine. Each symbol is the mean of 8 observations. Vertical lines show s.e.mean; where these are omitted, they are smaller than the symbol for the mean.

Increasing the concentration of atropine to 4×10^{-7}M produced a greater inhibition of contraction than that obtained with 1×10^{-7}M between 2 and 6 Hz but not at higher or lower frequencies (Figure 2b). Between 3 to 15 Hz, inhibition ranged from 51–55%. The 'notching' of the inhibition curve was not as apparent as with the lower concentration of atropine. A further increase in the concentration to 1×10^{-6}M reduced the inhibitory effect of atropine (Figure 2c). Inhibition over 3 to 15 Hz ranged from 29–55%. As with 1×10^{-7}M atropine, a 'notching' of the curve was apparent.

Tetrodotoxin (TTX) 5×10^{-7}M inhibited bladder contraction by between 80 and 90% at all frequencies employed (Figure 2d).

Effect of physostigmine, hexamethonium and physostigmine together with atropine on the excitatory response

The acetylcholinesterase inhibitor physostigmine (2×10^{-6}M) produced a slowly developing increase in tone which amounted to 1.95 ± 0.18 g (mean ± s.e.mean of 8 observations) by the end of the 30 min incubation period. Further, the normally small and infrequent spontaneous contractions were larger in amplitude and much more frequent when physostigmine was present. Both these effects were inhibited by atropine. Physostigmine (2×10^{-6}M) also enhanced the excitatory responses induced by field stimulation (Figure 3), especially at lower frequencies, an effect which diminished at higher frequencies as the maximum response of the tissue was reached. In combination with atropine, physostigmine did not greatly diminish the blockade of the former drug (Figure 3).

The ganglion-blocking agent, hexamethonium (1×10^{-4}M), significantly ($P < 0.05$) enhanced the contractile response between 4 and 15 Hz (Figure 3). This effect was abolished by guanethidine (1×10^{-6}M).

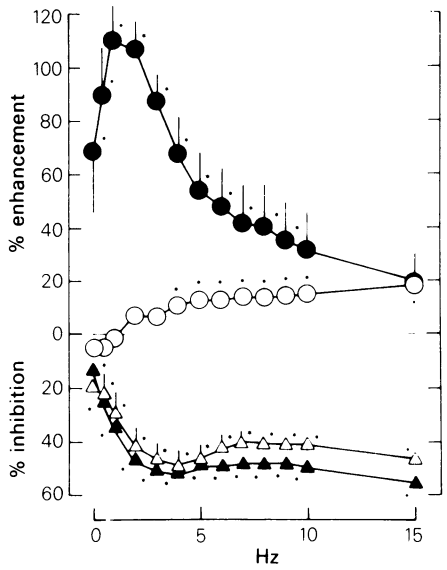

Figure 3 The effect of atropine, physostigmine, atropine plus physostigmine and hexamethonium on the electrically-induced contractions in guinea-pig isolated urinary bladder strips. Sixty min after the control curve (not shown) was obtained, either physostigmine (●, 2×10^{-6}M), atropine (▲, 4×10^{-7}M), or hexamethonium (○, 1×10^{-4}M) was added to the bath; after a 30 min incubation period, a second frequency-response curve was obtained in the presence of the drug. In the combination experiments (△) in which atropine (4×10^{-7}M) and physostigmine (2×10^{-6}M) were added together, the drugs were added 30 and 25 min, respectively, before the start of the second curve. Stimulation parameters were as described in Figure 1. Each symbol is the mean of 8 observations; vertical lines show s.e.mean. Effects are presented as a percentage change from the control (first) curve. Absolute responses obtained at each frequency in the presence and absence of drugs were compared statistically: $^*P < 0.05$.

Influence of adrenergic antagonists on excitatory responses

Since a portion of the field-stimulated motor response appeared to be non-cholinergically mediated, it was important to determine whether the adrenergic nervous system contributed to the contraction and whether it could account for some or all of the atropine-resistance.

The β-adrenoceptor antagonist, (±)-propranolol (5×10^{-6}M) and the α-adrenoceptor agonist, clonidine (3×10^{-8}M), significantly ($P < 0.05$) enhanced the contractile response at frequencies of 2–3 Hz or greater (Figure 4, Table 1). The α-

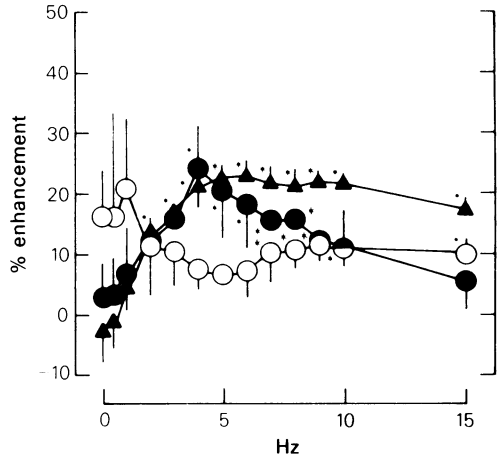

Figure 4 Influence of adrenergic antagonists on electrically-induced contractions in strips of guinea-pig isolated urinary bladder. (±)-Propranolol (●, 5×10^{-6}M), phentolamine (○, 5×10^{-6}M) and guanethidine (▲, 1×10^{-6}M) were each added to the bath 30 min before the second frequency-response curve and were present throughout. Stimulation parameters were as described in Figure 1. Each symbol is the mean of 8 observations; vertical lines show s.e.mean. Statistical comparisons were made as described in Figure 3. Effects are presented as a percentage change from the first (control) frequency-response curve: $^*P < 0.05$.

Table 1 The effect of clonidine on electrically-induced excitatory contractions of guinea-pig isolated urinary bladder[1]

Hz[2]	*Control*[2] (g)	*Clonidine* (3×10^{-8}M) (g)	% *enhancement*[5]	P[3]
0.1	3.44[4] ± 0.32	3.25 ± 0.39	−5.0 ± 7.0	> 0.05
0.5	4.45 ± 0.41	3.85 ± 0.47	−10.9 ± 10.9	> 0.05
1.0	5.50 ± 0.44	5.26 ± 0.59	−2.99 ± 9.49	> 0.05
2.0	8.56 ± 0.72	8.38 ± 1.07	−1.53 ± 9.62	> 0.05
3.0	11.8 ± 1.00	11.9 ± 1.44	0.9 ± 7.46	> 0.05
4.0	14.8 ± 1.32	15.4 ± 1.73	4.34 ± 6.88	> 0.05
5.0	16.9 ± 1.42	18.9 ± 1.90	12.0 ± 6.43	> 0.05
6.0	19.4 ± 1.47	21.2 ± 2.03	9.36 ± 4.83	> 0.05
7.0	20.6 ± 1.59	23.1 ± 2.13	12.3 ± 4.07	< 0.05
8.0	21.4 ± 1.65	24.7 ± 2.14	14.8 ± 2.83	< 0.05
9.0	21.8 ± 1.95	25.9 ± 2.23	20.0 ± 5.72	< 0.05
10.0	22.2 ± 1.99	26.4 ± 2.20	15.4 ± 4.32	< 0.05
15.0	27.0 ± 2.84	32.3 ± 2.96	21.5 ± 6.11	< 0.05

[1]Sixty minutes after the first (control) frequency-response curve clonidine (3×10^{-8}M) was added to the bath and 30 min later a second curve obtained in the presence of the agent.
[2]Stimulation parameters were supraximal voltage, 5 s trains, 0.5 ms duration at 1 min intervals.
[3]Level of significance.
[4]Values are mean ± s.e.mean of 8 observations.
[5]Minus sign signifies inhibition.

adrenoceptor antagonist, phentolamine (5×10^{-6}M), also enhanced the contractile response (Figure 4) at high frequencies ($P < 0.05$).

Guanethidine (1×10^{-6}M), an adrenergic neurone blocking agent, also enhanced bladder contractions at frequencies of 2 Hz or greater ($P < 0.05$, Figure 4). This enhancement was less dependent upon frequency than that seen with either (±)-propranolol or phentolamine. Higher concentrations of guanethidine (5×10^{-6}M) were no more effective.

The contribution of non-adrenergic non-cholinergic nerves to atropine-resistant responses

When tissues were exposed throughout the initial 90 min stabilization period to maximally effective concentrations of atropine (4×10^{-7}M), to remove the influence of the parasympathetic nerves and guanethidine (1×10^{-6}M) to inhibit sympathetic nerves, frequency-dependent contractions were still evident. For example, at a frequency of 15 Hz the maximal tension developed in the presence of these agents was 13.3 ± 0.95 g (mean ± s.e.mean, $n = 8$) compared to 29.1 ± 3.42 g ($n = 8$) obtained from untreated tissues prepared from weight matched animals.

The nature of the contraction obtained in the presence or in the absence of atropine and guanethidine differed greatly. In the absence of these agents, the initial contraction declined only slightly during the 5 s stimulus train and rapidly returned to baseline after stimulation ceased. On the other hand, in the presence of atropine and guanethidine, initial contractions declined rapidly in spite of continued stimulation yielding a 'spike-like' response. To determine whether responses obtained in the presence of atropine (4×10^{-7}M) and guanethidine (1×10^{-6}M) were reproducible with time, two frequency-response curves, 90 min apart, were obtained from bladder strips bathed in Krebs solution containing these drugs. When calculated as a percentage change from the control (first) curve, there appeared to be a small, but significant ($P < 0.05$), increase in tissue contractility when the frequency-response relationship was repeated 90 min later (Figure 5).

Tetrodotoxin (5×10^{-7}M), in the presence of atropine and guanethidine, significantly ($P < 0.05$) inhibited the response by 60 to 80% depending upon frequency (Figure 5). The inhibition produced by tetrodotoxin in the presence of atropine and guanethidine was calculated as a percentage change between the first and second frequency-response curves.

Tetrodotoxin-resistant responses

A portion of the response elicited by field stimulation was not susceptible to TTX and was presumably due to direct stimulation of the smooth muscle (Figures 2d and 5). However, a search for stimulation parameters that would yield responses which could be abolished at all frequencies by 5×10^{-7}M TTX (or higher) proved fruitless. At 0.2 ms responses were completely abolished by TTX but only at low fre-

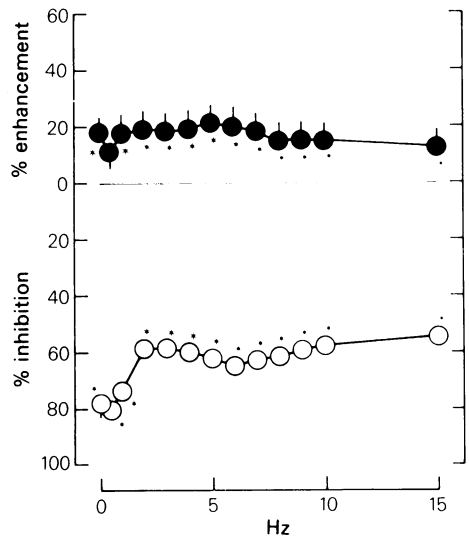

Figure 5 Reproducibility and effect of tetrodotoxin on non-adrenergic non-cholinergic excitatory responses in strips of guinea-pig isolated urinary bladder. Bladder strips were continuously bathed in Krebs solution containing atropine 4×10^{-7}M and guanethidine 1×10^{-6}M. In control experiments (●) two frequency-response curves separated by 90 min were obtained in each tissue. In another series of experiments, tetrodotoxin 5×10^{-7}M was added to the bath 30 min before constructing the second curve and was present throughout (○). Stimulation parameters were as described in Figure 1. Statistical comparisons were made as described in Figure 3. Each symbol is the mean of 8 observations; vertical lines show s.e.mean. Effects are presented as percentage changes from the control (first) curve: $*P < 0.05$.

quencies; at frequencies of about 2 or 3 Hz or greater a TTX-resistant component was present in some tissues. While this finding is identical to that of Downie & Dean (1977) in rabbit bladder, the TTX-resistant component was nevertheless a potentially complicating factor in the analysis of these data. Consequently, two types of experiments were performed to assess the persistence of the TTX-resistant component in the presence and absence of blocking agents. In the first, the TTX-resistant responses from two types of experiments were compared. In one set of tissues the first frequency-response curve was obtained in the absence of drugs. In a separate group, the first frequency-response curve was obtained in the presence of atropine (4×10^{-7}M) and guanethidine (1×10^{-6}M). Absolute maximal contractions at 15 Hz were 24.1 ± 3.92 and 13.3 ± 0.95 g ($P < 0.05$, $n = 8$), respectively. In both experiments, the second frequency-response curves were obtained in the presence of 5×10^{-7}M TTX (Table 2). The TTX-resistant contractions were identical even though the initial responses differed greatly, suggesting that the TTX-resistant portion of the contraction appears to be a relatively constant component.

In a second series of studies, the consistency of the TTX-resistant component with time was determined. Two frequency-response curves were obtained in the same tissues bathed in Krebs solution containing atropine (4×10^{-7}M) and guanethidine (1×10^{-6}M). TTX (5×10^{-7}M) was added to the bath 30 min before each curve and was present throughout. As exemplified by the contractions obtained at 15 Hz in the first, 4.72 ± 0.38 g, and second, 4.78 ± 0.38 g, curves ($P > 0.05$, $n = 6$), the TTX-resistant component appeared to remain constant over the course of 90 min. Hence, the increase in the response observed with time (Figure 5) cannot be explained by a change in smooth muscle responsiveness *per se*.

Discussion

Acetylcholine may not be the only neurotransmitter released from postganglionic neurones of the sacral parasympathetic nervous system. Organs innervated by this system, which appears to utilize an as yet unidentified neurotransmitter instead of, or, in addition to, acetylcholine, include: urinary bladder (see Introduction); anococcygeus muscle (Gillespie & McGrath, 1973; 1974; Creed & Gillespie, 1977); retractor penis (Luduena & Grigas, 1966; Klinge & Sjöstrand, 1974; Ambache & Killick, 1978) and gut (Ellis & Rasmussen, 1951; Crema, Tacca, Frigo & Lecchini, 1968; Burnstock, 1972). It has been suggested that the excitatory response of guinea-pig urinary bladder lacks a cholinergic involvement (Ambache & Zar, 1970) but present results indicate that cholinergic nerves do contribute to the excitatory responses induced by field stimulation.

Although atropine antagonized the electrically-induced motor responses at all frequencies, the maximum inhibition reached was only 55 to 60% and was considerably less below 3 Hz. At low to intermediate frequencies 4×10^{-7}M atropine was significantly ($P < 0.05$) more effective than either a lower (1×10^{-7}M) or, interestingly, a higher (1×10^{-6}M) concentration. The 'inflection' (Figure 2) in the atropine inhibition curve at 5 to 8 Hz has also been observed by Downie & Dean (1977) in rabbit detrusor muscle. These results clearly suggest that both an atropine-susceptible (presumably cholinergic) and an atropine-resistant component contribute to the field stimulated motor response in guinea-pig urinary bladder.

The involvement of the cholinergic nervous system was confirmed by studies with physostigmine which

Table 2 Comparison of tetrodotoxin (TTX)-resistant contractions of guinea-pig urinary bladder under differing experimental conditions[1]

	Control (g)	*Atropine + guanethidine* (g)	
Hz[2]	TTX 5×10^{-7}M	TTX 5×10^{-7}M	P[3]
0.1	0.48 ± 0.16[4]	0.80 ± 0.18	> 0.05
0.5	0.64 ± 0.21	0.78 ± 0.12	> 0.05
1.0	1.20 ± 0.38	1.26 ± 0.20	> 0.05
2.0	1.94 ± 0.54	2.61 ± 0.40	> 0.05
3.0	2.59 ± 0.68	3.14 ± 0.47	> 0.05
4.0	2.99 ± 0.78	3.62 ± 0.47	> 0.05
5.0	3.29 ± 0.84	4.05 ± 0.55	> 0.05
6.0	3.63 ± 0.91	4.20 ± 0.59	> 0.05
7.0	3.85 ± 0.92	4.59 ± 0.63	> 0.05
8.0	4.14 ± 0.99	4.86 ± 0.65	> 0.05
9.0	4.28 ± 1.00	5.11 ± 0.65	> 0.05
10.0	4.45 ± 1.05	5.21 ± 0.63	> 0.05
15.0	5.16 ± 1.19	6.10 ± 0.77	> 0.05

[1]Bladder strips were bathed in Krebs solution or in Krebs solution containing atropine (4×10^{-7}M) and guanethidine (1×10^{-6}M) for 90 min after which a frequency-response curve was constructed. The maximal contraction obtained in the first frequency-response curve in the absence or presence of atropine and guanethidine was 24.1 ± 3.92 and 13.3 ± 0.95 g ($P < 0.05$), respectively. After a 60 min washout TTX (5×10^{-7}M) was added followed 30 min later by a second curve obtained in the presence of the agent. Only the absolute contractions from the second curves are presented.

[2]Stimulation parameters were as described in Figure 1.

[3]Level of significance.

[4]Values are mean ± s.e.mean of 8 observations.

markedly potentiated responses to field stimulation. The enhancement was greatest at low frequencies (0.1 to 3 Hz), at which previous studies with atropine had demonstrated a comparatively minor cholinergic contribution to the total response. Thus, the small cholinergic contribution apparent in untreated strips at low frequencies is due to the enzymatic inactivation of acetylcholine. The inability of physostigmine to enhance the response when atropine was present suggests that the incomplete antagonism of responses by atropine is not the result of an inability to penetrate to sites of endogenous acetylcholine release, a view which confirms the observations of Ambache & Zar (1970).

Blockade of β-adrenoceptors with (±)-propranolol, inhibition of adrenergic neurotransmission with guanethidine, and reduction of noradrenaline release from sympathetic nerve endings via selective stimulation of prejunctional α-adrenoceptors with low concentrations of clonidine (Hieble & Pendleton, 1979) enhanced the contractile response. These effects confirm the view that sympathetic neurones are inhibitory to detrusor muscle via activation of β-adrenoceptors (Awad, Bruce, Carro-Ciampi, Downie, Lin & Marks, 1974; Dean & Downie, 1978; deGroat, Booth, Krier, Milne, Morgan & Nadelhaft, 1979; Levin, Shofer & Wein, 1980). The major role of the adrenergic innervation in bladder contraction appears to be inhibitory and therefore, does not account for atropine-resistant responses.

The enhancement of bladder contractions by phentolamine is more difficult to understand. The neck region of mammalian urinary bladder contains principally excitatory α-adrenoceptors with relatively fewer inhibitory β-receptors (Awad *et al.*, 1974; Levin *et al.*, 1980). Blockade of excitatory receptors, as well as antagonism of prejunctional α-adrenoceptors by phentolamine would be expected to yield an inhibition of the field stimulated response not, as was observed, an enhancement. A possible explanation might be that phentolamine is potentiating responses to the non-adrenergic non-cholinergic neurotransmitter, perhaps by blocking an inhibitory presynaptic α-receptor.

Bladder contractions occurring in the presence of maximally effective concentrations of atropine and guanethidine were characterized by: (1) a phasic component rather than the well maintained tonic contraction observed in the absence of these agents; (2) reaching a maximum at low frequencies; and (3) an apparent increase in tissue responsiveness with time. The first two correlate well with characteristics of the non-adrenergic non-cholinergic nervous system in other organs (Burnstock, 1972).

These findings suggest the presence of a non-adrenergic non-cholinergic nerve-mediated excitatory response which is maximum at low frequencies,

where a cholinergic contribution is minimal. Further, this component appears to be of importance in the initiation of bladder contraction rather than in the maintenance of tone.

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