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ADRENOCEPTORS OF THE GUINEA-PIG URINARY BLADDER

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1 Adrenaline, noradrenaline and isoprenaline (5 μg/ml) did not affect the resting tone of the isolated urinary bladder of the guinea-pig.

2 The catecholamines (1–2 μg/ml) inhibited neuronally evoked contractions at various stimulation frequencies; the inhibition was maximum at 2 Hz and minimum at 50 Hz. Isoprenaline produced maximum inhibition.

3 Propranolol (0.5 μg/ml) completely blocked the catecholamine-induced inhibition at all the frequencies employed. The concentration-response curves of isoprenaline at 2, 10 and 50 Hz were characteristically shifted by propranolol (50 ng/ml). Phenoxybenzamine (0.2 μg/ml) was totally ineffective.

4 In some experiments adrenaline significantly raised the tone of the bladder exposed to propranolol; this effect could be blocked by phenoxybenzamine.

5 Acetylcholine-induced bladder contractions were inhibited by adrenaline (2 μg/ml); the inhibition was completely blocked by propranolol (0.5 μg/ml).

6 The results indicate the presence of an inhibitory β-adrenoceptor and suggest the possibility of an excitatory α-adrenoceptor in guinea-pig urinary bladder.

Introduction

Edvardsen & Setekleiv (1968) failed to observe any response of isolated urinary bladder strips of guinea-pig to exogenous catecholamines, suggesting the lack of adrenoceptors in the guinea-pig bladder. However, using the same preparation De Sy (1970) showed that while the resting tone remained unaffected, the exogenous catecholamines inhibited the contractions evoked by supraluminal electrical stimulation, and the results with adrenoceptor blocking agents indicated the presence of inhibitory α- and β-adrenoceptors.

To our knowledge no further work has been done on the adrenoceptors in the guinea-pig bladder. The object of the present study was to characterize these adrenoceptors by observing the effect of catecholamines on the neuronally evoked bladder contractions *in vitro*. The isolated innervated urinary bladder preparation of the guinea-pig (Weetman, 1972) was found to be suitable for this purpose.

Methods

The nerve-bladder preparations obtained from adult guinea-pigs of either sex were set up as described by Weetman (1972) in a 30 ml organ bath containing Tyrode solution maintained at 35 ± 1°C and bubbled with 5% CO_2 in O_2. The Tyrode solution had the following composition (g/l): NaCl 8.0, KCl 0.2, $CaCl_2$ 0.2, $MgCl_2$ 0.1, NaH_2PO_4 0.05, $NaHCO_3$ 1.0 and glucose 1.0.

Contractions were recorded on a moving kymograph with isotonic frontal writing lever (load 1–1.5 g; magnification ×7). The bladders were neuronally stimulated with rectangular pulses (0.5 ms, 15–20 V) at different Hz for a variable duration so as to deliver 50–51 shocks every 2 minutes. Catecholamines have been reported to exhibit a frequency-dependent prejunctional inhibitory effect on cholinergic transmission (Vizi, 1968; Beani, Bianchi & Crema, 1969; Christ & Nishi, 1971; Knoll & Vizi, 1971). We, therefore, employed different frequencies (2 Hz–50 Hz) to distinguish a frequency-dependent effect, if any, of catecholamines on the guinea-pig bladder.

Each preparation served as its own control. The preparation was allowed to contract to neuronal stimulation for at least 20 min before the addition of any drug; the height of contraction was measured and was taken as the control. The contact period with the adrenoceptor blocking agent was 45 min and except for cumulative doses of isoprenaline, the tissue-catecholamine contact period did not exceed 4 minutes.

In a few experiments the effect of adrenaline on the

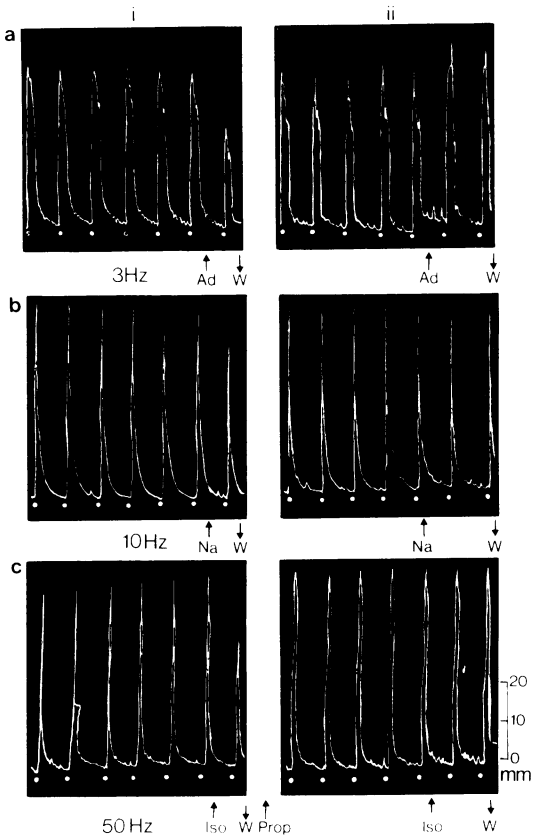

Figure 1 Isolated urinary bladder of guinea-pig; neuronal stimulation at dots (rectangular pulses, 15 V, 0.5 ms, 50–51 shocks every 2 min at frequencies indicated below). Effect of adrenaline (2 μg/ml in (a)), noradrenaline (2 μg/ml in (b)) and isoprenaline (1 μg/ml in (c)) added at Ad, Na and Iso respectively. Propranolol (0.5 μg/ml in (a) and (c), 0.2 μg/ml in (b)) added at Prop. Interval between panels (i) and (ii), 40 min; W = wash. Note the raised tone by adrenaline in (a) in presence of propranolol. Figure retouched for printing.

acetylcholine-induced contraction was recorded and then repeated in the presence of propranolol.

Drugs

(−)-Adrenaline acid tartrate (Sigma Chemicals, U.S.A.), (−)-noradrenaline acid tartrate (Fluka AG), isoprenaline sulphate (Burroughs Wellcome), propranolol hydrochloride (Inderal, ICI), phenoxybenzamine hydrochloride (SK & F) and acetylcholine bromide (Koch-Light Lab) were used and the concentrations refer to the salts.

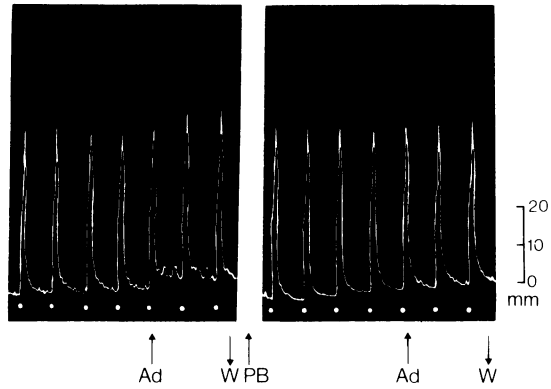

Figure 2 Isolated urinary bladder of guinea-pig in Tyrode solution containing propranolol (0.5 μg/ml). Effect of phenoxybenzamine (PB, 0.2 μg/ml) on adrenaline (Ad, 2 μg/ml)-induced rise in tone in the neuronally stimulated bladder (stimulation at dots, rectangular pulses, 15 V, 0.5 ms, 10 Hz for 50 shocks/2 minutes). Interval between the panels, 40 minutes. W = wash. Figure retouched for printing.

Results

Unless mentioned otherwise the number of experiments indicates experiments on separate bladders.

Effects of catecholamines on bladder tone and spontaneous rhythmic activity

The resting tone of the preparation was unaltered by adrenaline, noradrenaline or isoprenaline in concentrations up to 5 μg/ml (10 tests, 5 bladders). Following exposure to propranolol (0.2–0.5 μg/ml) 6 out of 15 bladders responsed to adrenaline (2 μg/ml) with a contraction averaging 3.8 ± 0.21 mm (mean ± s.e.) e.g. panel a(ii) of Figure 1). In these 6 preparations the mean contraction to adrenaline in the absence of propranolol was 0.8 ± 0.12 mm, the difference being statistically significant ($P < 0.001$, Student's t-test for paired data). Phenoxybenzamine (0.2 μg/ml) blocked this effect (Figure 2). In only one out of 10 experiments noradrenaline (2 μg/ml) raised the tone by 2.5 mm in the presence of propranolol. In 3 out of 6 experiments isoprenaline (1 μg/ml) reduced the tone slightly in the presence of phenoxybenzamine (0.2 μg/ml) but this was not statistically significant ($0.05 < P < 0.1$).

Spontaneous rhythmic activity, when present, was markedly reduced after isoprenaline although the tone remained unaffected. In a few experiments the spontaneous activity was increased after adrenaline in the presence of propranolol.

Table 1 Effects of catecholamines with and without propranolol (0.2–0.5 μg/ml) on the neuronally evoked contraction of the guinea-pig isolated urinary bladder (rectangular pulses, 15–20 V, 0.5 ms, 50–51 shocks/2 minutes)

		Height of contraction (mm)			*% inhibition by catecholamine*	
Catecholamine	*Frequency*	*Control*	*Catecholamine without propranolol*	*Catecholamine with propranolol (P > 0.1)*	*Without propranolol*	*With propranolol*
Adrenaline	2 Hz	19 ± 2 (9)	10 ± 1 (9)‡	17 ± 2 (7)	47	11
(2 μg/ml)	3 Hz	39 ± 3 (11)	26 ± 2 (11)†	35 ± 4 (8)	33	10
	10 Hz	49 ± 2 (27)	36 ± 2 (27)‡	47 ± 2 (21)	28	4
	50 Hz	51 ± 2 (15)	40 ± 1 (15)‡	50 ± 2 (12)	22	2
Noradrenaline	2 Hz	20 ± 2 (10)	11 ± 2 (10)†	18 ± 3 (7)	45	10
(2 μg/ml)	3 Hz	42 ± 4 (8)	29 ± 3 (8)*	44 ± 3 (6)	30	0
	10 Hz	50 ± 3 (9)	40 ± 3 (9)*	48 ± 3 (8)	20	4
	50 Hz	46 ± 3 (9)	38 ± 2 (9)*	46 ± 3 (7)	17	0
Isoprenaline	2 Hz	17 ± 2 (8)	7 ± 1 (8)‡	17 ± 2 (7)	59	0
(1 μg/ml)	10 Hz	44 ± 2 (14)	26 ± 2 (14)‡	40 ± 2 (13)	41	9
	50 Hz	49 ± 3 (15)	36 ± 2 (15)†	50 ± 4 (13)	27	0

Numbers in parentheses indicate number of tests on minimum 5 bladders.
Significance between control and catecholamine with and without propranolol was calculated using Student's *t* test: *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

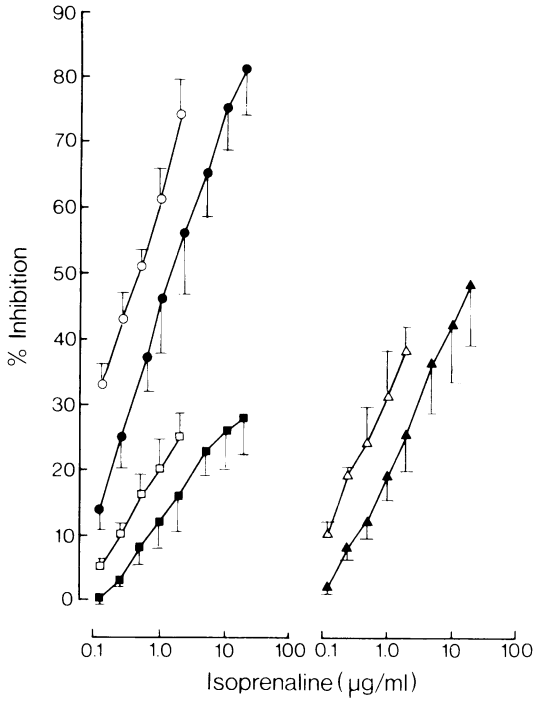

Figure 3 Isolated urinary bladder of guinea-pig. Cumulative concentration effect curves for the isoprenaline-induced inhibition of neuronally evoked contractions: 2 Hz, (○); 50 Hz (□); 10 Hz (△); rectangular pulses, 10–20 V, 0.5 ms; total shocks, 50; every 2 minutes. Isoprenaline effect is expressed as the percentage inhibition of the control responses. Propranolol (50 ng/ml) was incubated with the tissue for 45 min before repeating the inhibitory effect of isoprenaline at 2 Hz (●), 50 Hz (■), and 10 Hz (▲). Vertical lines show s.e. mean of six experiments.

Effects of catecholamines with and without adrenoceptor blocking agents (Table 1)

Adrenaline (2 μg/ml), noradrenaline (2 μg/ml) and isoprenaline (1 μg/ml) inhibited the neuronally evoked contractions in all the experiments and at all the stimulation frequencies. The inhibition was maximum at 2 Hz and minimum at 50 Hz. Maximum inhibition was produced by isoprenaline; adrenaline had a slightly greater inhibitory effect than noradrenaline.

Propranolol (0.2–0.5 μg/ml) and phenoxybenzamine (0.2 μg/ml) did not significantly affect the neuronally evoked contractions of the bladder. Irrespective of the frequency of stimulation propranolol (0.2–0.5 μg/ml) consistently and markedly blocked the adrenaline, noradrenaline and isoprenaline-induced inhibition (Figure 1). The blocking effect of propranolol persisted for at least 30 min after repeated washes.

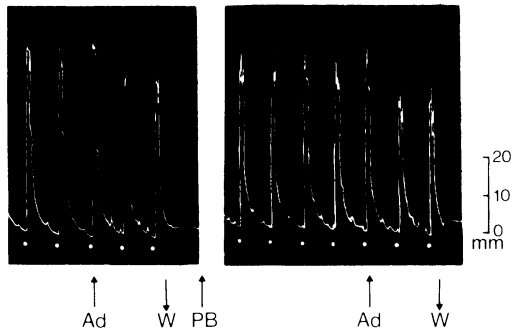

Figure 4 Isolated urinary bladder of guinea-pig. Effect of phenoxybenzamine (PB, 0.2 μg/ml) on the adrenaline (Ad, 2 μg/ml)-induced inhibition of the neuronally stimulated bladder (stimulation at dots, rectangular pulses, 15 V, 0.5 ms, 5 Hz for 50 shocks/2 minutes). Interval between the panels, 40 min, W = wash. Figure retouched for printing.

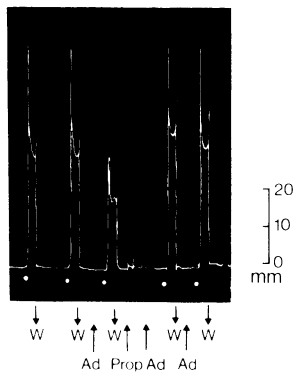

Figure 5 Isolated urinary bladder of guinea-pig. Effect of adrenaline (Ad, 2 μg/ml) on acetylcholine (1.5 μg/ml, added at dots)-induced contractions and its antagonism by propranolol (Prop, 0.5 μg/ml). Acetylcholine responses were repeated every 15 min except during incubation period with propranolol (45 minutes). Adrenaline was added 2 min before the next dose of acetylcholine. W = wash. Figure retouched for printing.

In 6 experiments cumulative dose-response curves were obtained for isoprenaline alone and in the presence of propranolol (50 ng/ml), at 2, 10 and 50 Hz frequencies. Propranolol antagonized the isoprenaline-induced inhibition at all the three frequencies (Figure 3).

Phenoxybenzamine (0.2 μg/ml) did not antagonize the adrenaline or noradrenaline-induced inhibition in any of the experiments (10 tests, 5 bladders); in 4 tests (2 bladders) the contractions were reduced further (Figure 4). The blockade produced by propranolol could not be increased further by phenoxybenzamine.

Effect of adrenaline on acetylcholine-induced contractions

Figure 5 shows the effect of adrenaline and its antagonism by propranolol on the acetylcholine-induced contraction of the guinea-pig bladder. In 5 tests on 3 bladders adrenaline (2 μg/ml) reduced the contraction produced by acetylcholine (1.5 μg/ml) to 55% (±3% s.e.) of the control; propranolol (0.5 μg/ml) antagonized this effect completely (the contraction reduced to 99% ± 1% s.e. of the control).

Discussion

The isolated urinary bladder of the guinea-pig is a ganglion-containing cholinergically innervated preparation (Weetman, 1972; Weetman & Turner, 1973). Adrenaline and noradrenaline have been shown to inhibit the release of acetylcholine through their prejunctional action on the α-adrenoceptor in the superior cervical ganglion of the rabbit (Christ & Nishi, 1971), in the guinea-pig ileum (Vizi, 1968; Knoll & Vizi, 1971) and the guinea-pig colon (Beani *et al.*, 1969): the inhibitory effect was evident at lower rather than at higher frequencies and could be blocked by α-adrenoceptor blocking agents. The prejunctional inhibition by noradrenaline varies inversely with the frequency and the total number of shocks delivered during stimulation; the fewer the shocks and the lower the frequency applied, the greater is the inhibition (Knoll & Vizi, 1971). The number of shocks (50–51/2 min) was kept constant in our experiments so that though the maximum inhibition by the catecholamines was produced at the low frequency (2 Hz), propranolol could block the inhibitory effect irrespective of the frequency of stimulation whereas phenoxybenzamine was totally ineffective. Though isoprenaline lacks the prejunctional inhibitory effect (Vizi, 1968) it was maximally inhibitory in the present experiments. These results plus the characteristic shift of the dose-response curves of isoprenaline by propranolol indicate that the inhibitory effect of adrenaline, noradrenaline and isoprenaline observed on the neuronally stimulated guinea-pig urinary bladder *in vitro* is produced by the action of the catecholamines on inhibitory β-adrenoceptors. Inhibition of the acetylcholine-induced contraction of the isolated bladder by adrenaline and its antagonism by propranolol provide evidence for the post-junctional site of this inhibitory effect of the catecholamines.

A slight but significant rise in the tone induced by adrenaline in the presence of propranolol was observed in a few experiments, and its blockade by phenoxybenzamine may suggest the existence of excitatory α-adrenoceptors in the guinea-pig bladder.

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