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THE OCCURRENCE OF POSTSYNAPTIC α- AND β-ADRENOCEPTORS IN THE GUINEA-PIG GALL BLADDER

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1 Guinea-pig gall bladder strips were contracted by (−)-noradrenaline, 10^{-5} M, and by field stimulation at 5 Hz (in the absence or presence of 10^{-6} M atropine) and relaxed to 10^{-5} M (−)-isoprenaline. (−)-Adrenaline, 10^{-5} M, predominantly contracted, but sometimes relaxed, this preparation.

2 In the presence of 10^{-6} M phentolamine, contractions to (−)-noradrenaline and to (−)-adrenaline were reversed to relaxations. The relaxations produced by (−)-isoprenaline were unaltered. In the presence of 10^{-6} M propranolol, contractions to (−)-noradrenaline increased in magnitude, relaxations to (−)-adrenaline were reversed to contractions, and relaxations to (−)-isoprenaline were abolished. These results demonstrate the presence of postsynaptic α-adrenoceptors which mediate contractions, and postsynaptic β-adrenoceptors which initiate relaxations, in the guinea-pig gall bladder.

3 The contractile responses to continuous field stimulation for 5 min at 5 Hz in Krebs solution alone were reduced in magnitude by propranolol, 10^{-6} M. In the presence of 10^{-6} M atropine (added to eliminate the cholinergic component of the response), propranolol, 10^{-6} M, had no effect on responses to stimulation at 5 Hz. Thus propranolol reduced the response to cholinergic stimulation in this tissue; the basis of this effect is unclear. In the absence or presence or atropine (10^{-6} M), the responses to 5 Hz were smaller in magnitude in the presence than absence of phentolamine, 10^{-6} M. This suggests that the responses to field stimulation of the guinea-pig gall bladder may, in part, be due to the release of endogenous noradrenaline which acts at postsynaptic α-adrenoceptors.

Introduction

The guinea-pig gall bladder receives a cholinergic (Davison & Fösel, 1975), a noradrenergic (Baumgarten & Lange, 1969; Davison, Al-Hassani, Crowe & Burnstock, 1978), and a non-adrenergic (inhibitory) innervation which may be 'purinergic' (Davison *et al.*, 1978). The presence of excitatory muscarinic cholinoceptors (Davison & Fösel, 1975) and of H_1 and H_2-histamine receptors, which mediate contraction and relaxation, respectively (Impicciatore, 1978), have been demonstrated clearly. Strips of guinea-pig gall bladder contract in response to (−)-noradrenaline and relax to (−)-isoprenaline (Doggrell & Scott, 1979). However, the adrenoceptors in the guinea-pig gall bladder have not been characterized. As a consequence, the role of the noradrenergic innervation of this tissue is unclear.

The present study was undertaken to characterize adrenoceptors in the guinea-pig gall bladder and to investigate the role of the noradrenergic innervation. The effects of the α- and β-adrenoceptor antagonists, phentolamine and propranolol, respectively (Furchgott, 1972) on the contractile response produced by exogenously-applied (−)-noradrenaline, (−)-isoprenaline, and (−)-adrenaline in the guinea-pig isolated gall bladder were examined. The effects of the same drugs on the responses of the preparation to field stimulation in the absence or presence of atropine were also investigated. Atropine was added to eliminate the excitatory cholinergic component of the response to stimulation (Davison & Fösel, 1975; Davison *et al.*, 1978).

A preliminary account of these findings has been presented to the British Pharmacological Society (Doggrell & Scott, 1980).

Methods

Male or female adult guinea-pigs were killed by cervical dislocation and the gall bladder removed and cut into 4 approximately equal longitudinal strips. All experiments were performed in a modified Krebs sol-

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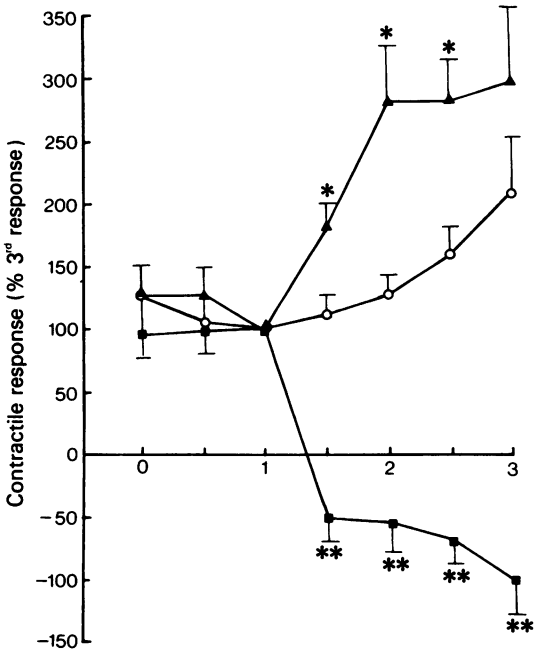

Figure 1 The effects of phentolamine and propranolol on the responses of the guinea-pig gall bladder to (−)-noradrenaline, 10^{-5} M: (○) in Krebs solution, (■) when phentolamine, 10^{-6} M, was added to the Krebs solution following the third response, and (▲) when propranolol, 10^{-6} M, was added to the Krebs solution following the third response. All responses are expressed as a percentage of the third response in Krebs solution (ordinate scale) and plotted against time (abscissa scale). Each value is the mean from 7 to 9 preparations; vertical lines show s.e. mean. $*P < 0.05$; $**P < 0.0005$. Contractions to (−)-noradrenaline, 10^{-5} M, were converted to relaxations in the presence of phentolamine, 10^{-6} M, and increased in magnitude in the presence of propranolol, 10^{-6} M.

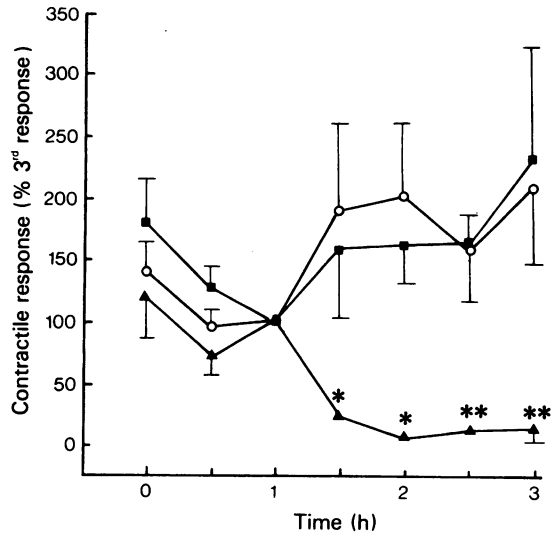

Figure 2 The effects of phentolamine and propranolol on the relaxant responses of the guinea-pig gall bladder produced by (−)-isoprenaline, 10^{-5} M: (○) in Krebs solution, (■) when phentolamine, 10^{-6} M, was added to the Krebs solution following the third response, and (▲) when propranolol, 10^{-6} M, was added to the Krebs solution following the third response. All responses are expressed as a percentage of the third response in Krebs solution (ordinate scale) and plotted against time (abscissa scale). Each value is the mean from 6 to 10 preparations; vertical lines show s.e. mean. $*P < 0.025$; $**P < 0.0005$. Relaxations to (−)-isoprenaline, 10^{-5} M, were unaltered in the presence of phentolamine, 10^{-6} M, and abolished in the presence of propranolol, 10^{-6} M.

ution (composition, mM: NaCl 116, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 22.0, D-glucose 11.2 and Na_2EDTA, 0.04) equilibrated with 5% CO_2 in O_2, at 37°C.

Strips were mounted between 2 platinum electrodes, approximately 8 cm apart, under 1 g tension in 5 ml organ baths containing Krebs solution. This tension was maintained throughout the experiment by regular adjustment. The tissues relaxed for 1 to 1 h 45 min before maintaining the 1 g tension and were thus equilibrated for 1 h 45 min. Tissues were then continuously stimulated electrically (5 Hz biphasic pulses of 1 ms duration and supramaximal voltage) or exposed to agonists for 5 min. The tissues were then allowed to recover and a period of 25 min (the first 20 min representing a time when the tissues were washed by overflow) was allowed to elapse before the next stimulation or addition of agonist. Contractile responses were recorded isometrically with force displacement transducers (Grass model FT03.C) connected to a polygraph (Grass model 79B).

When atropine, 10^{-6} M, was used, it was added to the Krebs solution from the beginning of the equilibration period. Time-dependent changes in the sensitivity of strips of guinea-pig gall bladder to field stimulation at 5 Hz (in the absence or presence of 10^{-6} M atropine) and to exogenously applied (−)-noradrenaline, 10^{-5} M, or isoprenaline, 10^{-5} M, have been demonstrated (Doggrell & Scott, 1979). Thus when studying the effect of phentolamine or propranolol on contractile responses, experiments were carefully controlled to allow for time-dependent changes. Phentolamine or propranolol (10^{-6} M) was added to the Krebs solution bathing one strip of guinea-pig gall bladder 1 h after the first control response was completed while another strip remained in Krebs solution throughout the experiment.

All responses were expressed as a percentage of the third response in Krebs solution (i.e. the response

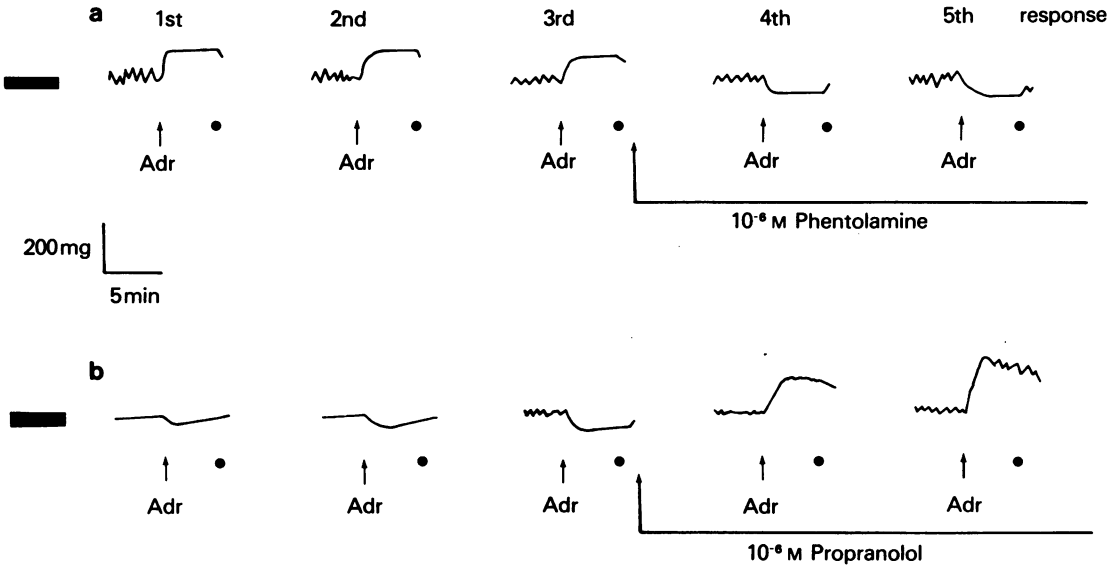

Figure 3 Responses of the guinea-pig gall bladder to (−)-adrenaline (Adr), 10^{-5} M. (a) A tissue that contracted to 10^{-5} M (−)-adrenaline in Krebs solution. This contraction was reversed to a relaxation on the addition of phentolamine, 10^{-6} M. (b) A tissue that relaxed to 10^{-5} M (−)-adrenaline in Krebs solution. This relaxation was reversed to a contraction in the presence of propranolol 10^{-6} M. ■ = Resting tension level of 1 g; (●) = wash.

obtained 1 h after the first response was initiated). This response was called, nominally, the 100% or control response. The values obtained under different conditions, were compared using Student's *t*-test, and differences were considered to be significant when $P < 0.05$.

The drugs used were: phentolamine mesylate (donated by Ciba), (−)-adrenaline bitartrate, atropine sulphate, (−)-isoprenaline bitartrate, (−)-noradrenaline bitartrate, (±)-propranolol hydrochloride, and tetrodotoxin (Sigma).

Results

Effects of (−)-noradrenaline, (−)-isoprenaline, (−)-adrenaline and field stimulation

(−)-Noradrenaline, 10^{-5} M, consistently contracted strips of guinea-pig gall bladder (32 strips from 8 animals tested). On the addition of 10^{-5} M (−)-isoprenaline, 33 of 40 strips relaxed; the others were unaffected. (−)-Adrenaline, 10^{-5} M, contracted 31 out of 56 preparations, relaxed 15 preparations from 9 out of 14 animals, and produced both contractions and relaxations in 4 preparations. (−)-Adrenaline had no effect in the remaining 6 preparations. Strips of guinea-pig gall bladder contracted in response to continuous field stimulation for 5 min at 5 Hz (32 preparations from 8 animals). Contractions to 5 Hz were also observed in the presence of 10^{-6} M atropine (44 of 48 preparations). Similar time-dependent changes to those previously described by Doggrell & Scott (1979) were observed with all the agents which induced contractile responses. Thus the magnitude of the contractile responses to 5 Hz (in the absence or presence of 10^{-6} M atropine), to (−)-noradrenaline, 10^{-5} M, and to (−)-adrenaline, 10^{-5} M each increased with time. The second relaxant response to (−)-isoprenaline, 10^{-5} M, was smaller than the first; the relaxant responses then increased with time.

Effects of phentolamine and propranolol

The contractile responses to (−)-noradrenaline, 10^{-5} M, were converted to relaxations in the presence of 10^{-6} M phentolamine (Figure 1). In the presence of propranolol (10^{-6} M), the magnitude of contractions produced by (−)-noradrenaline, 10^{-5} M, increased (Figure 1). The relaxant responses to (−)-isoprenaline, 10^{-5} M, were unaltered by 10^{-6} M phentolamine and inhibited by 10^{-6} M propranolol (Figure 2). In tissues that contracted to (−)-adrenaline, 10^{-5} M, alone, relaxations in the presence of 10^{-6} M phentolamine in all 7 strips tested (representing 7 animals) were observed. In tissues that relaxed to 10^{-5} M (−)-adrena-

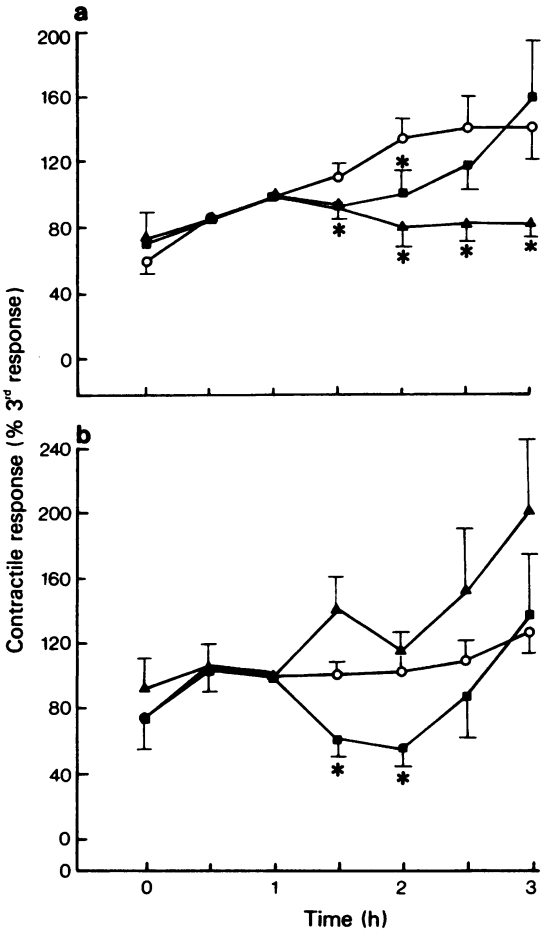

Figure 4 The effects of phentolamine and propranolol on the responses of the guinea-pig gall bladder to continuous field stimulation at 5 Hz for 5 min (biphasic pulses of 1 ms duration, supramaximal voltage). In (b) all solutions contained atropine 10^{-6} M; in (a) there was no atropine present. (○) Responses in Krebs solution; (■) when phentolamine, 10^{-6} M, was added to the Krebs solution following the third response, and (▲) when propranolol, 10^{-6} M was added to the Krebs solution following the third response. All responses are expressed as a percentage of the third response in Krebs solution (ordinate scale) and plotted against time (abscissa scale). Each value is the mean from 8 to 12 preparations; vertical line show s.e. mean. $*P < 0.05$.

line, the responses were reversed to contractions in the presence of 10^{-6} M propranolol (preparations from 6 animals). Representative responses to 10^{-5} M (−)-adrenaline are shown in Figure 3.

In Krebs solution alone, contractions to field stimulation at 5 Hz were not altered by a 30 min period of incubation with phentolamine 10^{-6} M (Figure 4). Following a 1 h incubation, the responses to 5 Hz were smaller in the presence of 10^{-6} M phentolamine. The responses to 5 Hz after a 1.5 or 2 h period of incubation with phentolamine, 10^{-6} M, were of the same magnitude as responses in Krebs solution alone. In Krebs solution alone, the magnitude of the contractile responses to 5 Hz was smaller in the presence of 10^{-6} M propranolol after 0.5, 1, 1.5 or 2 h of incubation. In the presence of 10^{-6} M atropine (Figure 4), the contractile responses to 5 Hz were smaller in magnitude following a 30 min or 1 h incubation in the presence of 10^{-6} M phentolamine; this effect was not observed after a 1.5 or 2 h incubation. Propranolol, 10^{-6} M, had no effect on the responses to continuous field stimulation for 5 min at 5 Hz obtained in the presence of 10^{-6} M atropine.

Discussion

In isolated strips of guinea-pig gall bladder time-dependent changes following application of certain stimuli (Doggrell & Scott, 1979) are observed. The contractile responses to field stimulation (in the absence or presence of atropine), to (−)-noradrenaline, to acetylcholine and to ATP increase in magnitude with time. The magnitude of the relaxant responses to (−)-isoprenaline initially decreases and then increases with time. Doggrell & Scott (1979) have suggested that these time-dependent changes are prostaglandin-mediated and emphasized the necessity of carefully controlling experiments using this preparation. In the present study, three responses were obtained in each tissue in the absence of phentolamine or propranolol. Phentolamine or propranolol was then added to the medium bathing one strip of guinea-pig gall bladder while another strip remained untreated. This experimental design allows drug-induced changes to be distinguished.

Adrenoceptors in the guinea-pig gall bladder have not been characterized previously. There are two pharmacological procedures for the characterization of adrenoceptors (see review by Furchgott, 1972). First, the relative potencies of a series of adrenoceptor agonists in eliciting a specific response may be determined. (−)-Noradrenaline acts predominantly at α-adrenoceptors, (−)-adrenaline can elicit responses at α- and β-adrenoceptors, and (−)-isoprenaline is solely a β-adrenoceptor agonist. In the present study, (−)-noradrenaline contracted strips of guinea-pig gall bladder, (−)-adrenaline caused contractions and relaxations, and (−)-isoprenaline mediated relaxations. These results suggest the presence of postsynaptic α-adrenoceptors, that mediate contractions, and postsynaptic β-adrenoceptors, that initiate relaxations, in the guinea-pig gall bladder.

The second procedure for adrenoceptor characterization is the use of antagonists. Responses mediated by α-adrenoceptors are antagonized by phentolamine while propranolol is an antagonist at all β-adrenoceptors. In the present study the contractions produced by (−)-noradrenaline and (−)-adrenaline were reversed to relaxations by phentolamine. At the concentration used (10^{-6} M), it seems likely that phentolamine acts solely at α-adrenoceptors as the responses to (−)-isoprenaline were unaffected. The relaxant responses to (−)-isoprenaline were abolished by propranolol. These studies with specific α- and β-antagonists support the presence of postsynaptic α-(mediating contractions) and β-(relaxations) adrenoceptors in the guinea-pig gall bladder. Similar results have been obtained using the cat gall bladder (Persson, 1972).

In the presence of an α-adrenoceptor antagonist (phentolamine), (−)-noradrenaline and (−)-adrenaline relaxed gall bladder strips. This preparation was contracted by these agents in the presence of a β-adrenoceptor blocker (propranolol). Thus it seems likely that in the absence of antagonists, (−)-noradrenaline and (−)-adrenaline act at both α- and β-adrenoceptors, the overall contractile response observed being the net effect of contraction and relaxation.

The responses to continuous field stimulation of gall bladder strips were abolished and reduced, respectively, by tetrodotoxin and atropine (Doggrell & Scott, 1979). This confirms the excitatory cholinergic innervation to the guinea-pig gall bladder (Davison & Fösel, 1975). The nature of the residual excitatory innervation is unknown. In the absence of atropine, propranolol was a potent inhibitor of responses to field stimulation i.e. of the cholinergic excitatory response. The inhibitory action of propranolol may be due to its β-adrenoceptor blocking or its local anaesthetic action; this has not been resolved. In the presence of atropine, propranolol had no effect on responses to field stimulation. Thus we have no evidence that β-adrenoceptors are involved in the response to non-cholinergic nerve stimulation.

The responses to continuous field stimulation in the absence or presence of atropine were reduced, to a small extent, by phentolamine. Thus it seems likely that responses to field stimulation in the guinea-pig gall bladder may, in part, be due to a release of endogenous noradrenaline which acts at postsynaptic α-adrenoceptors.

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