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ACCUMULATION AND OVERFLOW OF ^{3}H FOLLOWING INCUBATION OF THE GUINEA-PIG GALL BLADDER WITH [^{3}H]-NORADRENALINE

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1 Strips of guinea-pig gall bladder readily accumulate ^{3}H following incubation in the presence of 5×10^{-8} M (−)-[^{3}H]-noradrenaline. This accumulation was reduced by lowering the incubation temperature (from 37° to 23°C), by cocaine (10^{-6} M), by nortriptyline (10^{-8}, 10^{-6} and 10^{-4} M) and following incubation of the tissues with 6-hydroxydopamine (10^{-3} M for 3 h). At 10^{-6} M, (−)-noradrenaline and (−)-adrenaline, but not (−)-isoprenaline, inhibited the accumulation of ^{3}H.

2 Following preloading of strips of guinea-pig gall bladder with 3.6×10^{-7} M (−)-[^{3}H]-noradrenaline for 1 h, the spontaneous overflow of ^{3}H was observed. Cocaine (10^{-4} M), nortriptyline (10^{-6} M), (−)-isoprenaline (10^{-5} M), acetylcholine (10^{-5} M) and adenosine 5′-triphosphate (ATP, 10^{-4} M) had no effect on the spontaneous overflow of ^{3}H. KCl (10^{-1} M), (−)-noradrenaline (10^{-5} M), (−)-adrenaline (10^{-5} M), and tyramine (10^{-5} M) increased the overflow of ^{3}H. These results illustrate similar characteristics of the guinea-pig gall bladder to other noradrenergically-innervated tissues in accumulating and releasing ^{3}H following incubation in the presence of [^{3}H]-noradrenaline.

3 Following incubation in the presence of 3.6×10^{-7} M (−)-[^{3}H]-noradrenaline, field stimulation, at 5 Hz, of strips of gall bladder, in the absence or presence of 10^{-6} M atropine, increased the overflow of ^{3}H and, simultaneously, induced contractions. The contractile responses to 5 Hz were smaller in the presence than in the absence of 10^{-4} M lignocaine. Lignocaine (10^{-4} M) reduced the overflow of ^{3}H evoked by field stimulation at 5 Hz. It is suggested that the contractile responses to 5 Hz are due to nerve stimulation and that the increased overflow of ^{3}H is due to the stimulation of noradrenergic nerves.

4 The overflow of ^{3}H evoked by field stimulation at 5 Hz was unaltered and increased by propranolol (10^{-6} M) and phentolamine (10^{-6} M), respectively. Clonidine (5×10^{-5} M) had no effect in the absence but reduced the amount of ^{3}H which overflowed in response to field stimulation at 5 Hz in the presence of 10^{-6} M atropine. The contractile responses to field stimulation at 5 Hz were reduced by phentolamine (10^{-6} M) or clonidine (5×10^{-6}, 10^{-5} and 5×10^{-5} M) whether or not atropine (10^{-6} M) was present. These results illustrate the presence of postsynaptic α-adrenoceptors and suggest the presence of presynaptic α-adrenoceptors in the gall bladder of the guinea-pig.

Introduction

The gall bladder of the guinea-pig receives an excitatory 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 nature of the noradrenergic innervation is unclear. Contractions of strips of guinea-pig gall bladder produced by field stimulation (in the presence of atropine) or on addition of (−)-noradrenaline are antagonized by phentolamine (Doggrell & Scott, 1980). This suggests that the nerve-mediated excitatory contractions may, in part, be due to the release of endogenous noradrenaline which acts at postsynaptic α-adrenoceptors.

In the present study the ability of the guinea-pig gall bladder to accumulate ^{3}H following incubation in the presence of (−)-[^{3}H]-noradrenaline has been demonstrated and the accumulation and overflow of (−)-[^{3}H]-noradrenaline which occurred spontaneously or in response to field stimulation studied.

Methods

Mature Dunkin Hartely guinea-pigs of either sex were killed by cervical dislocation and the gall bladder was removed. All experiments were performed in the presence of a modified Krebs solution of the following

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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 disodium edetate (Na_2EDTA) 0.04, equilibrated with 5% CO_2 in O_2, at 37°C.

Determination of (−)-[^{3}H]-noradrenaline accumulation

Each gall bladder was divided into 6 approximately equal pieces, weighing 4 to 6 mg each. The individual pieces were mounted on a wire frame in 10 ml Krebs solution and equilibrated for 15 min. (−)-[^{3}H]-noradrenaline, final concentration 5×10^{-8} M, was added for the appropriate length of time and then the tissues were blotted and washed for 10 min in 10 ml Krebs solution. The tissues were then digested in 'Protosolve' (120 g NaOH in 1 l methanol) and a toluene based scintillation fluor added. The tritium in the tissue and the medium was determined by liquid scintillation spectrometry. Quenching was corrected for by the external standards method. Tissue:medium ratios were calculated.

When studying the effects of cocaine, nortriptyline, or sympathomimetics on noradrenaline accumulation, different concentrations of these drugs were added to the Krebs solution 5 min before incubation with (−)-[^{3}H]-noradrenaline. When investigating the effect of 6-hydroxydopamine on accumulation, the isolated tissues were incubated in the presence and absence of 6-hydroxydopamine for 3 h before the noradrenaline accumulation experiment was begun.

Overflow of ^{3}H following preloading with (−)-[^{3}H]-noradrenaline

Each gall bladder was divided into 4 approximately equal longitudinal pieces. The individual pieces were mounted on a wire frame in 20 ml Krebs solution and equilibrated for 15 min. (−)-[^{3}H]-noradrenaline, final concentration 3.6×10^{-7} M, was added for 1 h. At the end of 1 h the strips were treated as follows:

(i) *Spontaneous overflow.* Each strip was transferred to 3 ml of fresh Krebs solution. The replacement of Krebs solution was repeated at 5 min intervals for 2 h.

(ii) *Effect of drugs on spontaneous overflow.* Each tissue was placed in 30 ml of prewarmed Krebs solution for 1 h 15 min. The strips were then transferred to 3 ml of fresh Krebs solution and this solution was replaced at 5 min intervals for 30 min. When studying the effects of drugs on spontaneous overflow, these agents were added after 1 h 25 min of overflow for the final 20 min.

(iii) *Field stimulation-induced overflow.* Each strip was placed in 30 ml of Krebs solution for 1 h. Within 15 min, each tissue was mounted under 1 g tension in 5 ml organ baths between 2 platinum electrodes. The Krebs solution was replaced at 5 min intervals for 85 min. The tissues were stimulated at 5 Hz (1 ms duration, supramaximal voltage) for 5 min after 1 h 25 min, 2 h, and 2h 35 min of release. Contractile responses were measured isometrically with force displacement transducers (Grass Model FTO3.C) and recorded on a polygraph (Grass Model 79B).

When experiments were performed in the presence of atropine, 10^{-6} M atropine was present in the Krebs solution after 1 h of release. When studying the effect of lignocaine, propranolol, phentolamine or clonidine on field stimulation-evoked overflow, these agents were added to the Krebs solution, after the first stimulation period, for the final 70 min of release.

The amount of tritium remaining in the tissue and that in the medium were determined by liquid scintillation spectrometry. Overflow was expressed as the percentage overflow as follows: % overflow = $A/A' \times 100$, where A = amount of ^{3}H that overflowed in a 5 min period and A′ = amount of ^{3}H in the tissue at the beginning of the overflow period.

Contractile responses were expressed as a percentage of the response obtained after 1 h 25 min of overflow. This response was called, nominally, the 100% or control response.

The values obtained under different conditions, were compared by Student's *t*-test, and differences were considered to be significant when $P < 0.05$.

(−)-[^{3}H]-noradrenaline with a specific activity of 2.2 Ci/mmol was obtained from the New England Nuclear Corporation. The other drugs used were lignocaine hydrochloride (Astra Pharmaceuticals Ltd), clonidine hydrochloride* (Boehringer Ingelheim), phentolamine mesylate* (Ciba), nortriptyline hydrochloride* (Lilly), cocaine hydrochloride (May & Baker) and acetylcholine chloride, adenosine 5′-triphosphate (ATP), (−)-adrenaline bitartrate, atropine sulphate, 6-hydroxydopamine hydrochloride, (−)-isoprenaline bitartrate, (−)-noradrenaline bitartrate, (±)-propranolol hydrochloride and tyramine hydrochloride (Sigma Chemicals Ltd.). Compounds indicated with an asterisk were generously donated by the companies shown.

Results

Time course of ^{3}H accumulation

Following incubation in the presence of 5×10^{-8} M (−)-[^{3}H]-noradrenaline, the guinea-pig gall bladder readily accumulated ^{3}H (Figure 1). Accumulation was observed within 5 min and the highest tissue:medium ratio of 6.87 ± 1.36 (mean ± s.e. mean, $n = 6$) was obtained after 15 min.

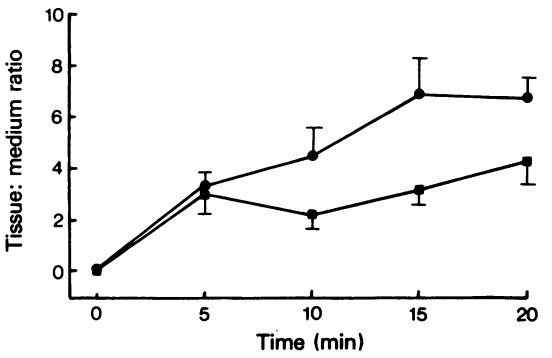

Figure 1 Time course of the accumulation of radioactivity in the guinea-pig gall bladder following incubation with 5×10^{-8} M (−)-[^{3}H]-noradrenaline: at 37°C (●), and at 23°C (■). ^{3}H accumulation was calculated as the tissue:medium ratio (ordinate scale). Abscissa scale:time (min). Each value is the mean from 5–7 preparations; vertical lines show s.e. mean.

Effect of temperature on ^{3}H accumulation

The ability of the guinea-pig gall bladder to accumulate ^{3}H was temperature-sensitive. At 23°C, the accumulation of ^{3}H was lower, following incubation for 10, 15, or 20 min in the presence of 5×10^{-8} M (−)-[^{3}H]-noradrenaline, than at 37°C (Figure 1).

Effect of drugs on ^{3}H accumulation

The accumulation of (−)-noradrenaline, within a 10 min incubation period, was inhibited by cocaine, 10^{-6}, 10^{-4} M, and by nortriptyline, 10^{-8}, 10^{-6}, 10^{-4} M (Figure 2). Following preincubation in the presence of 6-hydroxydopamine, 10^{-3} M, for 3 h, the ability of the tissue to accumulate (−)-noradrenaline, over a 10 min period, was greatly reduced in comparison to that of tissues preincubated in Krebs solution alone (Figure 2). At a final concentration of 10^{-6} M, (−)-noradrenaline and (−)-adrenaline, but not (−)-isoprenaline, inhibited the accumulation of (−)-[^{3}H]-noradrenaline (Figure 2). At 10^{-4} M, (−)-isoprenaline also inhibited noradrenaline accumulation.

Spontaneous overflow (Figure 3)

After incubation of the guinea-pig gall bladder for 1 h in the presence of 3.6×10^{-7} M (−)-[^{3}H]-noradrenaline, there was a rapidly declining overflow of ^{3}H for the first 20 min followed by a period during which the overflow declined slowly (20 to 70 min). After 70 min, no further significant reduction in overflow was observed.

Effect of drugs on the spontaneous overflow of ^{3}H

The effect of drugs on the spontaneous overflow of ^{3}H was studied after 85 min of overflow, when spontaneous overflow was no longer declining. In the presence of KCl, 10^{-1} M, the overflow of ^{3}H from the guinea-pig gall bladder was increased (Figure 4). Cocaine, 10^{-4} M, and nortriptyline, 10^{-6} M, had no effect on spontaneous overflow (Figure 4). In the presence of a high concentration of nortriptyline, 10^{-4} M, the overflow of ^{3}H was increased (Figure 4). ^{3}H overflow from the guinea-pig gall bladder was also increased by (−)-noradrenaline, 10^{-5} M, (−)-adrenaline, 10^{-5} M and tyramine, 10^{-5} M, (Figure 4). (−)-Isoprenaline, 10^{-5} M, (Figure 4), acetylcholine, 10^{-5} M, and ATP, 10^{-4} M, were ineffective.

Field stimulation-induced release of ^{3}H and contractile responses

After 85 min of spontaneous overflow and subsequently at 120 and 150 min, field stimulation at 5 Hz (5 min duration), in the absence or presence of 10^{-6} M atropine, increased the overflow of ^{3}H (Figure 5) and simultaneously induced contractions of strips of guinea-pig gall bladder. The ^{3}H overflow evoked by 5 Hz in each time period was not significantly different. Contractile responses to 5 Hz, in the absence or presence of 10^{-6} M atropine, increased in magnitude with time (Figure 6).

On the addition of lignocaine 10^{-4} M, propranolol 10^{-6} M, phentolamine 10^{-6} M, or clonidine 10^{-6}, 5×10^{-6}, 10^{-5} and 5×10^{-5} M, after the first stimulation period, no significant difference was observed in spontaneous overflow of ^{3}H compared to that in the absence of these agents. The effects of these agents on the field stimulation-evoked increase in overflow were investigated. Lignocaine (10^{-4} M), in the absence or presence of 10^{-6} M atropine, reduced the overflow of ^{3}H obtained by field stimulation at 5 Hz (Figure 5). The magnitude of the contractile responses evoked by field stimulation at 5 Hz was smaller in the presence than the absence of 10^{-4} M lignocaine (Figure 6a). Propranolol (10^{-6} M) had no effect on the overflow of ^{3}H evoked by field stimulation (5 Hz, 5 min duration) in the absence or presence of atropine. In Krebs solution alone, the contractile responses to 5 Hz were smaller in the presence than the absence of 10^{-6} M propranolol (Figure 6b). 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 5 Hz (Figure 6b).

Phentolamine (10^{-6}M), in the absence or presence of 10^{-6} M atropine, increased the overflow of ^{3}H at 5

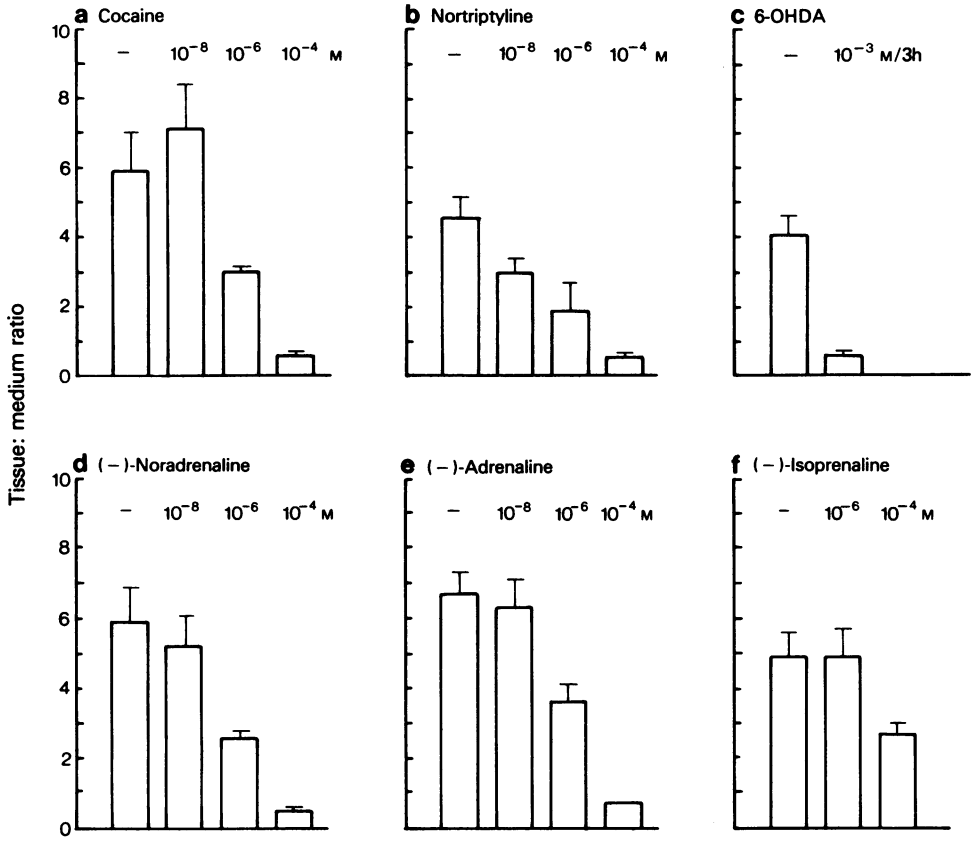

Figure 2 The effect of cocaine (a), nortriptyline (b), 6-hydroxydopamine incubation (c), (−)-noradrenaline (d), (−)-adrenaline (e) and (−)-isoprenaline (f) on (−)-[^{3}H]-noradrenaline accumulation in the guinea-pig gall bladder. ^{3}H accumulation was calculated as the tissue:medium ratio (ordinate scale). Each value is the mean from 4 to 5 preparations following a 10 min incubation in the presence of 5×10^{-8} M (−)-[^{3}H]-noradrenaline; vertical lines show s.e. mean.

Hz (Figure 7). The magnitude of the contractile responses to 5 Hz, in the absence or presence of 10^{-6} M atropine, was smaller in the presence than absence of 10^{-6} M phentolamine (Figure 6c). In the absence or presence of 10^{-6} M atropine, clonidine (10^{-6} M, 5×10^{-6} M and 10^{-5} M) had no effect on the overflow of ^{3}H evoked by field stimulation at 5 Hz. In Krebs solution alone, 5×10^{-5} M clonidine, also, had no effect on the overflow of ^{3}H (Figure 8). Clonidine (5×10^{-5} M) reduced the overflow of ^{3}H evoked by field stimulation at 5 Hz in the presence of 10^{-6} M atropine (Figure 8). Clonidine (10^{-6} M) had no effect on the contractile responses to 5 Hz. In the absence or presence of 10^{-6} M atropine, the contractile responses to 5 Hz were smaller in the presence than in the absence of 5×10^{-6}, 10^{-5} and 5×10^{-5} M (Figure 6d) clonidine.

Discussion

There are two main processes by which noradrenaline can be taken up by tissues; neuronal ($Uptake_1$) and extraneuronal ($Uptake_2$) uptake. Neuronal uptake of [^{3}H]-noradrenaline is temperature-sensitive (reviewed by Paton, 1976), inhibited by cocaine and tricyclic antidepressant drugs e.g. nortriptyline (reviewed by

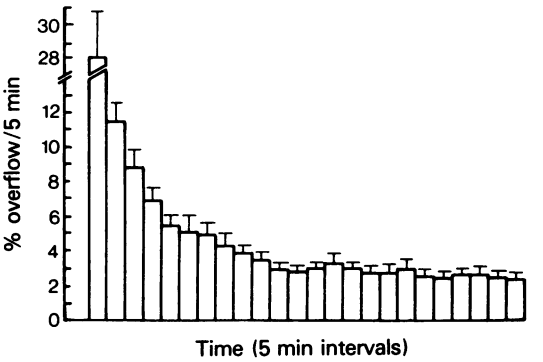

Figure 3 The spontaneous overflow of ^{3}H from the guinea-pig gall bladder following incubation with 3.6×10^{-7} M (−)-[^{3}H]-noradrenaline. Overflow is expressed as % overflow/5 min (ordinate scale). Abscissa scale: time. Each column is the mean from 4 preparations; vertical lines show s.e. mean.

Maxwell, Ferris & Burcsu, 1976), reduced by 6-hydroxydopamine incubation (Doggrell & Woodruff, 1978), and potently inhibited by unlabelled noradrenaline and adrenaline, but not isoprenaline (reviewed by Ross, 1976). In the present study, following incubation in the presence of 5×10^{-8} M [^{3}H]-noradrenaline, the accumulation of ^{3}H in the guinea-pig gall bladder had all of these characteristics. Thus it seems likely that following incubation with this low concentration of noradrenaline, neuronal accumulation of noradrenaline is observed in guinea-pig gall bladder.

Following incubation in the presence of [^{3}H]-noradrenaline, the spontaneous overflow of ^{3}H measured in the present study is equal to the release minus the reuptake of ^{3}H. At a time when spontaneous overflow was not declining, it seems unlikely that reuptake was a major determinant of overflow, as cocaine, at a concentration that almost abolished neuronal uptake (10^{-4} M), had no effect on spontaneous overflow. Thus agents which increased spontaneous overflow did so by increasing release. KCl, unlabelled (−)-noradrenaline, (−)-adrenaline and tyramine released ^{3}H from this tissue. As a consequence, any spasmogenic activity of these agents on the guinea-pig gall bladder may be due, in part, at least, to the release of endogenous noradrenaline. A high concentration of nortriptyline (10^{-4} M), which caused a similar degree of inhibition of neuronal uptake to 10^{-4} M cocaine, also increased the spontaneous overflow of ^{3}H. Thus high concentrations of nortriptyline may release endogenous noradrenaline from this tissue, an effect not reported with other noradrenergically innervated tissues. It is unlikely that this agent could induce spasmogenic activity as nortriptyline also possesses marked α-adrenoceptor blocking activity at this concentration (Doggrell & Woodruff, 1977).

Strips of guinea-pig gall bladder contract in the presence of acetylcholine or ATP and relax on the addition of (−)-isoprenaline (Doggrell & Scott, 1979). The present study demonstrates that following preloading of this tissue with [^{3}H]-noradrenaline, acetylcholine, ATP and (−)-isoprenaline do not release ^{3}H. Thus contractile responses to these agents are not due to the release of endogenous noradrenaline.

Following preloading of the guinea-pig gall bladder with [^{3}H]-noradrenaline, field stimulation increased the release of ^{3}H, an effect reduced in the presence of lignocaine. Local anaesthetics e.g. lignocaine, block noradrenergic transmission (reviewed by Häusler & Haefely, 1979). Thus it seems likely that field stimulation evoked release of ^{3}H from noradrenergic nerves.

Accompanying the nerve-evoked release of ^{3}H, contractions of strips of guinea-pig gall bladder were observed. Although the ^{3}H overflow in each time period was similar, the contractile responses to 5 Hz, in the absence of presence of atropine, increased in magnitude with time. This confirms an earlier study (Doggrell & Scott, 1979); an increased overflow of ^{3}H does not form the basis of this effect.

The contractile responses of strips of guinea-pig gall bladder to field stimulation are reduced by atropine (Doggrell & Scott, 1979). This illustrates the excitatory cholinergic innervation of this tissue previously demonstrated by Davison & Fösel (1975). The contractions produced by field stimulation, in the presence of atropine or on addition of (−)-noradrenaline are antagonized by phentolamine (Doggrell & Scott, 1980). This suggests that the residual excitatory response in the presence of atropine is noradrenergic. Recently Doggrell & Scott (1980) found that 10^{-6} M propranolol reduced the magnitude of the contractile responses to field stimulation in this tissue in the absence but not the presence of atropine. This ability of propranolol to reduce the cholinergic excitatory response is confirmed by the present study. Propranolol has β-adrenoceptor blocking and local anaesthetic activity (Prichard, 1978). It seems unlikely that the effect of propranolol observed in this study is related to its local anaesthetic action as propranolol did not affect the release of ^{3}H from noradrenergic nerves. This suggests that propranolol acts at a β-adrenoceptor to reduce the cholinergic excitatory response.

The nerve-evoked release of noradrenaline is regulated by presynaptic adrenoceptors (reviewed by Starke, 1977; Langer, 1979). Thus in the presence of low concentrations of noradrenaline i.e. in the range of low frequencies of nerve stimulation, presynaptic β-adrenoceptors are stimulated, leading to an increase

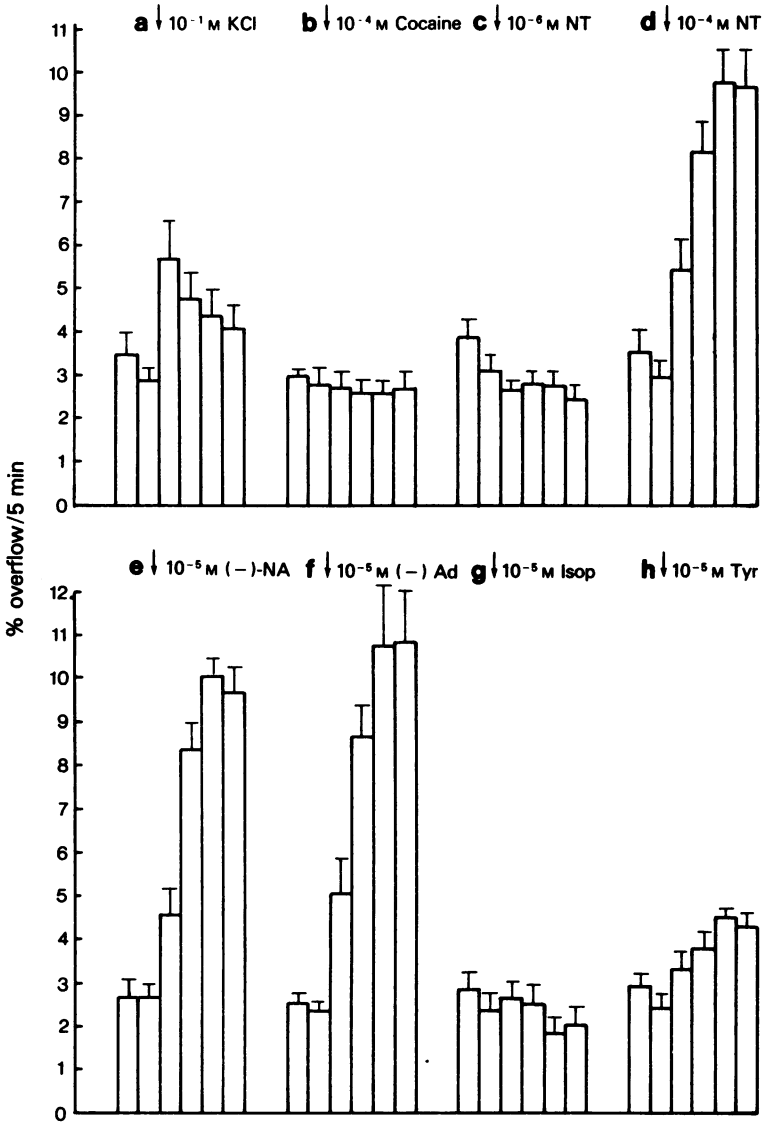

Figure 4 The effects of KCl (a), cocaine (b), nortriptyline (NT) 10^{-6} M (c), nortriptyline 10^{-4} M (d), (−)-noradrenaline (NA) (e), (−)-adrenaline (Ad) (f), (−)-isoprenaline (Isop) (g), and tyramine (Tyr) (h) on the spontaneous overflow of ^{3}H from the guinea-pig gall bladder following incubation in the presence of [^{3}H]-noradrenaline. Overflow is expressed as % overflow/5 min (ordinate scale). Each value is the mean from 4 to 9 preparations; vertical lines show s.e. mean.

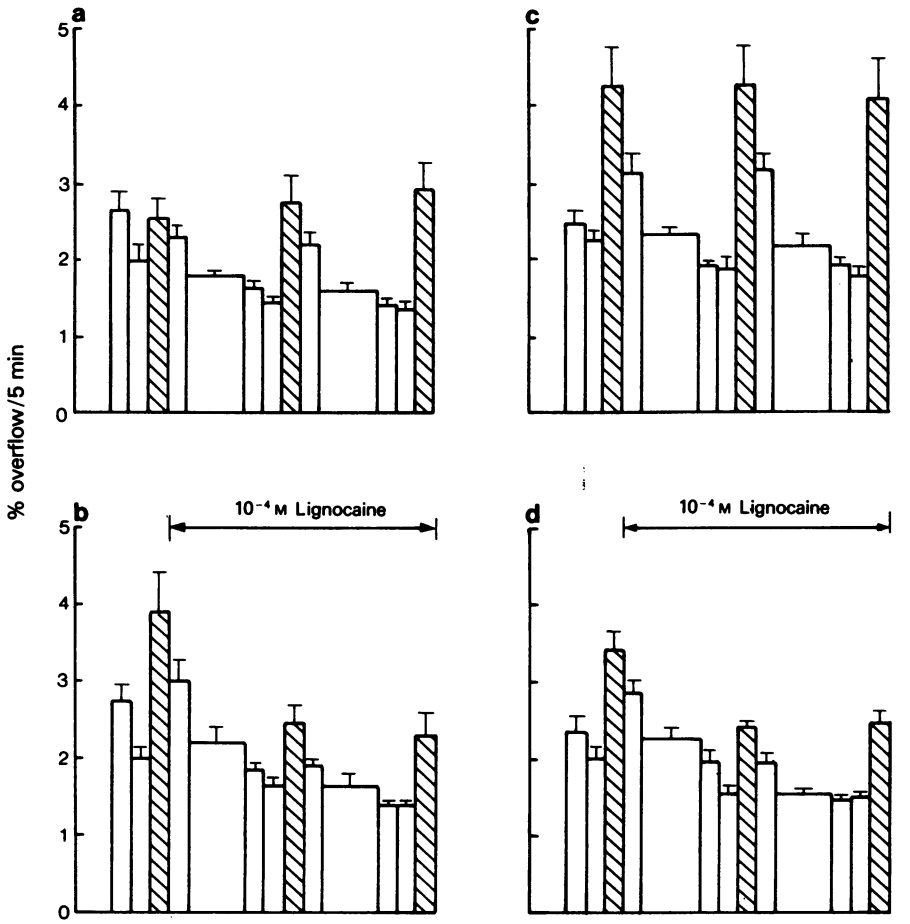

Figure 5 The overflow of ^{3}H from the guinea-pig gall bladder following incubation with [^{3}H]-noradrenaline: in the absence (a and b) and presence (c and d) of atropine. At hatched columns, tissues were stimulated electrically (5 Hz, 1 ms duration, supramaximal voltage) for 5 min. In (b) and (d), lignocaine (10^{-4} M) was added after the first response to 5 Hz. Overflow is expressed as % overflow/5 min (ordinate scale). Each value is the mean from 6 preparations.

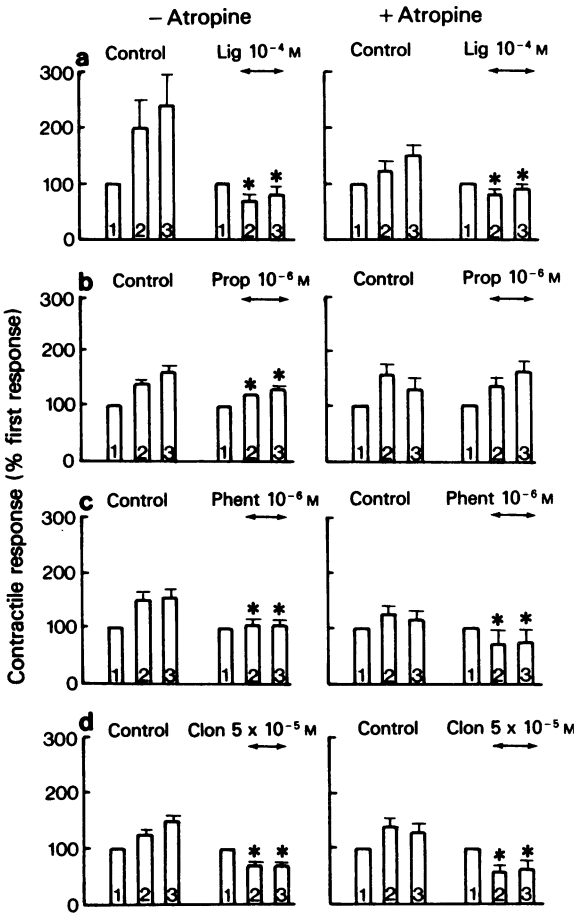

Figure 6 The effect of lignocaine (Lig) 10^{-4} M (a), propranolol (Prop) 10^{-6} M (b), phentolamine (Phent) 10^{-6} M (c) and clonidine (Clon) 5×10^{-5} M (d), on contractile responses to field stimulation at 5 Hz (5 min duration) in strips of guinea-pig gall bladder. Left panel: without atropine; right panel: in the presence of 10^{-6} M atropine. Contractile responses are expressed as a percentage of the first response (ordinate scale). Each value is the mean result from 5–6 preparations; vertical lines show s.e. mean. $^{*}P < 0.05$, paired t-test.

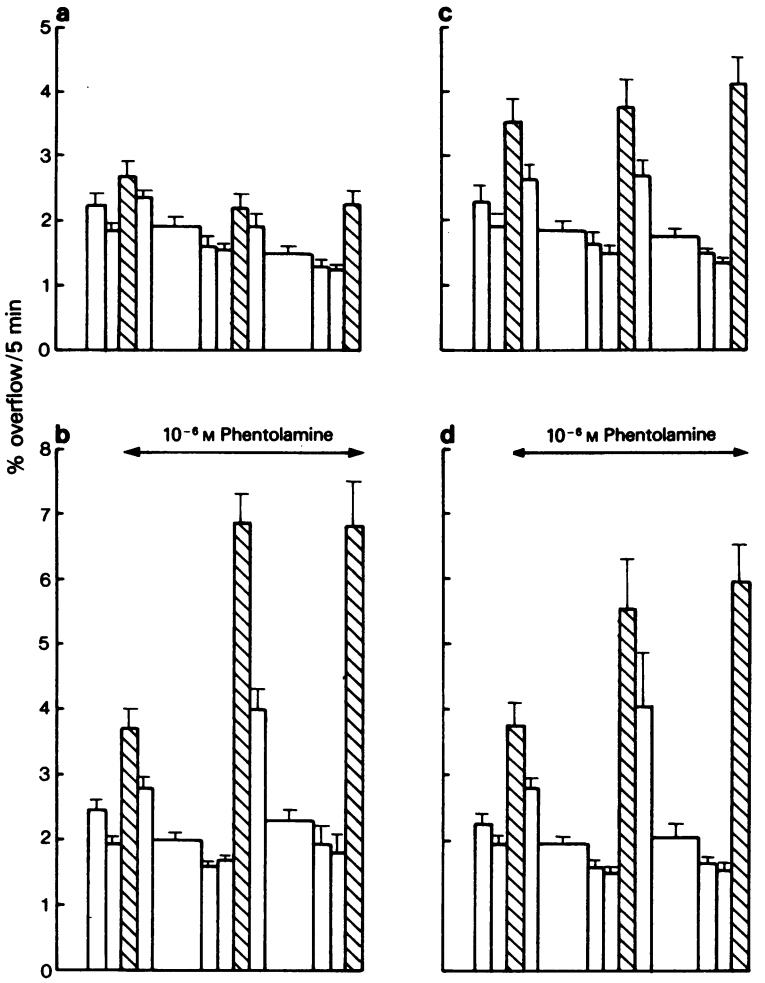

Figure 7 The overflow of ^{3}H from the guinea-pig gall bladder following incubation with [^{3}H]-noradrenaline in the absence (a and b) and presence (c and d) of 10^{-6} M atropine. At hatched columns tissues were stimulated electrically (5 Hz, 1 ms duration, supramaximal voltage) for 5 min. In (b) and (d), phentolamine (10^{-6} M) was added after the first response to 5 Hz. Overflow is expressed as % overflow/5 min (ordinate scale). Each value is the mean result from 6 preparations; vertical lines show s.e. mean.

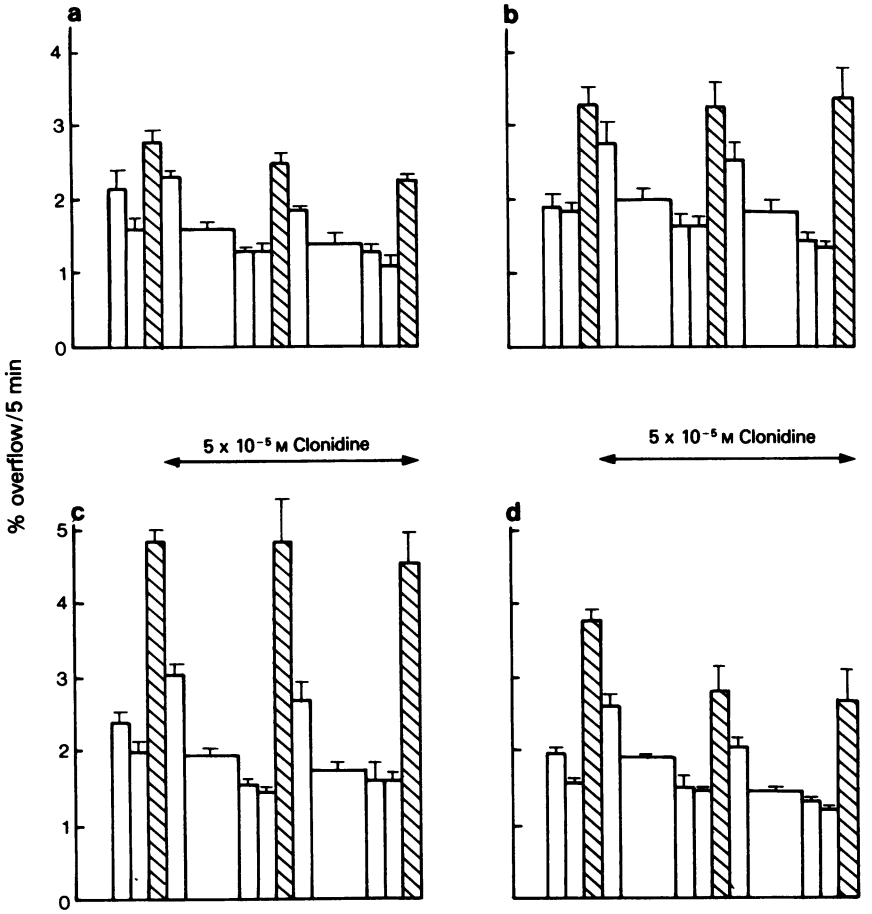

Figure 8 The overflow of ^3H from the guinea-pig gall bladder following incubation with [^3H]-noradrenaline in the absence (a and b) and presence (c and d) of 10^{-6} M atropine. At hatched columns tissues were stimulated electrically (5 Hz, 1 ms duration, supramaximal voltage) for 5 min. In (b) and (d), clonidine (5×10^{-5} M) was added after the first response to 5 Hz. Overflow is expressed as % overflow/5 min (ordinate scale). Each value is the mean result from 5 preparations; vertical lines show s.e. mean.

in transmitter release. When higher concentrations of noradrenaline are reached in the synaptic cleft, the regulatory mechanism mediated by presynaptic α-adrenoceptors is triggered. Stimulation of these receptors reduces transmitter release. In the present study, 10^{-6} M propranolol had no effect on the release of ^3H evoked by field stimulation at 5 Hz. Thus, with this concentration of propranolol and at 5 Hz, no evidence of presynaptic β-adrenoceptor activation was observed. This does not eliminate the possibility that with other concentrations of propranolol or at lower frequencies of nerve stimulation presynaptic β-adrenoceptors may be demonstrated in this tissue.

Presynaptic α-adrenoceptors have been demonstrated in all noradrenergically innervated tissues so far studied (reviewed by Starke, 1977). The nerve-evoked release of ^3H from the guinea-pig gall bladder was increased by phentolamine, an α-adrenoceptor antagonist (Furchgott, 1972). Clonidine is an α-adrenoceptor agonist which acts preferentially at presynaptic α-adrenoceptors (Starke, Montel, Gay & Merker, 1974). Under one set of conditions used in the present study i.e. 5×10^{-5} M clonidine in the presence of atropine, clonidine decreased the release of ^3H at 5 Hz. Thus it seems likely that the guinea-pig gall bladder contains presynaptic α-adrenoceptors.

As well as acting at presynaptic α-adrenoceptors to increase nerve-evoked release of ^{3}H, phentolamine at the same time reduced the magnitude of the contractile responses to 5 Hz. The latter action of phentolamine may be due to antagonism at postsynaptic α-adrenoceptors. Clonidine (5×10^{-6} and 10^{-5} M) had no effect on the release of ^{3}H but reduced the contractile responses to 5 Hz. This ability of clonidine to reduce contractile responses may be related to its partial agonistic activity which allows it to antagonize responses to noradrenaline at postsynaptic α-adrenoceptors (Medgett, McCulloch & Rand, 1978).

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