Possible mechanisms of inhibition with atropine against noradrenaline-induced contraction in the rabbit aorta

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1 In the rabbit isolated aorta, atropine (3×10^{-6} M– 10^{-4} M) inhibited contractile response to noradrenaline without affecting contraction to KCl.

2 In the presence of contraction to noradrenaline, atropine (3×10^{-7} M– 10^{-4} M) caused concentration-dependent relaxation. Pretreatment with theophylline (10^{-3} M) potentiated the relaxant action of atropine. Relaxation to atropine was not affected by the specific guanosine 3':5'-cyclic monophosphate phosphodiesterase inhibitor, M & B 22,948 (10^{-4} M), tetraethylammonium (10 mM), indomethacin (10^{-5} M), propranolol (10^{-7} M), nifedipine (10^{-6} M) or removal of the endothelium.

3 Relaxation to either atropine or prazosin was not affected by preincubation with prazosin and atropine, respectively.

4 In Ca^{2+} -free medium containing EGTA and nifedipine, atropine (10^{-7} M– 10^{-4} M) inhibited the residual noradrenaline response more than the subsequent Ca^{2+} -induced contraction. Pretreatment with either theophylline (10^{-3} M), forskolin (3×10^{-7} M) or a low concentration of prazosin (3×10^{-9} M) also inhibited the residual contraction to noradrenaline and Ca^{2+} . The effect of combined treatment of atropine and any of these agents was much greater than with each individual agent.

5 Atropine (10^{-6} M– 10^{-4} M) also inhibited increases in the level of inositol monophosphates (IP) in response to noradrenaline. Theophylline (10^{-3} M) and a low concentration of prazosin (3×10^{-9} M) also inhibited IP formation. Combined with atropine, the effect was much greater than with each of these agents individually.

6 Atropine did not affect adenosine 3':5'-cyclic monophosphate (cyclic AMP) levels in the aorta and also failed to displace specific [^3H]-prazosin binding.

7 These results suggest the possibility that smooth muscle relaxation to atropine may be due to the inhibition of phosphoinositide metabolism. The relaxation is not apparently due to an action of atropine on α_1 -adrenoceptors, or a change in the level of cyclic AMP.

Keywords: Atropine; anti-cholinoceptor agents; adrenoceptors; vascular smooth muscle; noradrenaline; endothelium; inositol phosphate (IP)

Introduction

It is well known that atropine (($\pm$)-hysocamine) is a competitive antagonist at acetylcholine-muscarinic receptor sites in smooth muscles and other tissues (see Katzung, 1987). A high concentration of atropine was reported to antagonize contraction of smooth muscle strips by noradrenaline in cattle (Denac *et al.*, 1991). It was also reported to inhibit the increased motility caused by cholecystokinin octapeptide in the jejunum and colon of dogs (Fargeas *et al.*, 1989) and abolish adenosine-induced increases in histamine contraction in canine bronchial smooth muscles (Sakai *et al.*, 1989). Recently we also found that atropine, but not scopolamine ((-)-hyoscine) and other muscarinic receptor antagonists, caused relaxation of noradrenaline-induced contractions in vascular smooth muscles of rabbit. Based on these observations, the present study was conducted to define the mechanism of the inhibitory effect of atropine on the response to noradrenaline in vascular tissue.

Methods

Rabbit aorta: isolation, preparation, and protocol

Male New Zealand white rabbits weighing 2.5–3.0 kg were killed by an air injection through an ear vein during ether

anaesthesia. The thoracic aorta was isolated, excess fat and connective tissue removed, and then cut into cylindrical segments which were subsequently cut open. Preparations (approximately 3–4 mm in width and 10–13 mm in length) were mounted vertically in organ baths containing 20 ml of Krebs solution of the following composition (mM): NaCl 120.3, KCl 4.8, CaCl_2 1.2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.3, KH_2PO_4 1.2, NaHCO_3 24.2 and glucose 5.8 at pH 7.4. The tissue bath solution was maintained at 37°C and bubbled with a 95% O_2 and 5% CO_2 gas mixture. Ca^{2+} -free solution was prepared by omitting CaCl_2 from the previously described solution and by including EGTA (0.01 mM) and nifedipine (10^{-6} M). Ligatures were placed around both ends of the muscle strips, one attaching the muscle to a glass holder and the other to a transducer. The preparation was adjusted to give an initial resting tension of 2 g. Changes in isometric tension were recorded through a force displacement transducer (FT-03) connected to a six-channel Grass polygraph. The contractile responses were measured as increases above rest tension. Concentration-response curves were performed in a cumulative manner. When antagonists were used, tissues were pretreated with an antagonist 10 min before the addition of an agonist. The endothelium was removed from some preparations by rubbing the intimal surface of the aorta with wet filter paper. In these preparations, acetylcholine (3×10^{-7} M) failed to relax the contraction induced by noradrenaline (3×10^{-7} M). The response in the absence of inhibitor was taken as 100%.

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Measurement of inositol monophosphate

The accumulation of [^3H]-inositol monophosphate (IP) was determined by a slight modification of the procedure of Brown & Brown (1983) and Rapoport (1986). Briefly, rings of rabbit aorta were incubated at 37°C for 3 h in Krebs solution containing [^3H]-inositol ($4\ \mu\text{Ci}\ 0.5\ \text{ml}^{-1}$ per ring). The tissues were transferred to Krebs solution containing 10 mM LiCl and placed on ice for 30 min. Noradrenaline ($10^{-5}\ \text{M}$) and other drugs were added to the solution and the mixture was incubated at 37°C for an additional 60 min. Each tissue was then homogenized in 1.0 ml of 10% trichloroacetic acid. The homogenates were centrifuged at 300 g for 15 min and the supernatants were extracted 3 times with 2.0 ml of ether; 4 ml of 5 mM NaHCO_3 was added to 1 ml of the aqueous phase and the mixture was transferred to a column containing anion exchange resin (Bio-Rad AGI-X8, 100–200 mesh, formate form). The column was washed with 10 ml water to remove [^3H]-inositol. [^3H]-inositol monophosphate was eluted with 6 ml 200 mM ammonium formate in 100 mM formic acid.

Scintillation cocktail (Aquasol-2, New England Nuclear) was added to the eluate and the samples were counted in a liquid scintillation counter.

[^3H]-prazosin binding

Aortae were thawed in ice-cold 0.32 M sucrose. Each aorta was placed on a glass plate on an ice bath, kept moist with 0.32 M sucrose and the surrounding fat carefully trimmed, avoiding any mechanical damage to the tissue. The tissue was then crosschopped with a McIlwain tissue chopper (Surrey, England) at 300 μm and homogenized in a Brinkman Polytron (Kinematica, Switzerland) at setting 7, single burst for 30 s. The homogenate was subjected to two successive centrifugations at 1,000 g and 45,000 g for 15 and 30 min, respectively. The pellet was resuspended in 0.05 M Tris buffer pH 8.0 containing 1 mM MgCl_2 , using the polytron at setting 7 for 30 s, and centrifuged at 45,000 g for 20 min. The final pellet was resuspended in Tris buffer at a protein concentration of 1.0–1.2 mg ml^{-1} . [^3H]-prazosin binding was assayed in the aortic membrane preparation as established earlier in our laboratory (Simmons & Jones, 1988). Membrane protein concentration of 0.20 to 0.25 mg per assay tube was found to produce optimum results with 60 min incubation. Specific binding was determined over a ligand concentration range of 0.1 to 10 nM and was defined as the difference between [^3H]-prazosin bound in the absence and presence of $10^{-5}\ \text{M}$ phentolamine. Protein was assayed by the method of Lowry *et al.* (1951) with bovine serum albumin used as standard.

Cyclic AMP measurement

Each rabbit aortic ring was preincubated in 1 ml of Krebs-Ringer solution for 1 h at 37°C before the incubation with atropine ($10^{-6}\ \text{M}$ – $10^{-4}\ \text{M}$) for 20 min; 1 ml of 12% cold trichloroacetic acid was then added and the tissue was homogenized at 4°C. The homogenate was centrifuged at 1700 g for 10 min and the supernatant was extracted 3 times with 3 ml of water-saturated ether. Adenosine 3':5'-cyclic monophosphate (cyclic AMP) in the supernatant (100 μl) was measured by radioimmunoassay (Amersham cAMP [^{125}I] assay system, RPA.509).

Statistical analysis

Data are presented as the means $\pm$ s.e.mean and statistical analysis was performed with a Student's *t* test or Analysis of Variance followed by Dunnett's test. Significant differences between control and test groups were evaluated with $P < 0.05$ as the level of significance.

Drugs

The following chemicals were used: noradrenaline bitartrate, phentolamine, atropine sulphate salt, prazosin HCl, scopolamine, gallamine, forskolin, tetraethylammonium chloride, pirenzepine (all Sigma); propranolol HCl (Ayerst Lab); theophylline (Matheson, Coleman & Bell); indomethacin (Merk, Sharp & Dohme); nifedipine (Pfizer); M & B 22,948 (2-O-propoxyphenyl-8-azapurine-6-one; May & Baker); myo-[2- ^3H (N)]-inositol (specific activity $15.6\ \text{Ci}\ \text{mmol}^{-1}$) and [^3H]-prazosin ([^3H]-PRZ; specific activity $18.1\ \text{Ci}\ \text{mmol}^{-1}$) (New England Nuclear); cyclic AMP [^{125}I] assay system (Amersham).

Results

In rabbit isolated aorta, atropine ($10^{-5}\ \text{M}$ and $10^{-4}\ \text{M}$) inhibited contractile responses to noradrenaline ($10^{-9}\ \text{M}$ – $3 \times 10^{-6}\ \text{M}$) (Figure 1a). Specifically, atropine at $10^{-5}\ \text{M}$ and $10^{-4}\ \text{M}$ shifted the concentration-response curve for noradrenaline to the right without significantly altering the maximal contraction (Figure 1a). However, atropine at $10^{-4}\ \text{M}$ did not cause any reduction or shift in the response to KCl (10 mM–70 mM) (Figure 1b).

Following contraction of the aorta with noradrenaline ($3 \times 10^{-7}\ \text{M}$), atropine ($10^{-6}\ \text{M}$ – $10^{-4}\ \text{M}$) caused relaxation in a concentration-dependent manner (Figure 2). Scopolamine ($10^{-4}\ \text{M}$), pirenzepine ($10^{-4}\ \text{M}$) and gallamine ($10^{-4}\ \text{M}$) had only a negligible effect on the noradrenaline ($3 \times 10^{-7}\ \text{M}$)-contracted aorta (scopolamine: $11.3 \pm 3.4\%$ reduction, $n = 4$; pirenzepine: $3.1 \pm 1.7\%$ reduction, $n = 5$; gallamine: $4.2 \pm 2.3\%$ reduction, $n = 5$). Pretreatment of the aorta with theophylline ($10^{-3}\ \text{M}$) markedly potentiated the relaxant action of atropine, although M & B 22,948 ($10^{-4}\ \text{M}$) did not have any significant effect on the relaxation (Figure 2). Similarly, pretreatment of the aorta with tetraethylammonium (TEA; 10 mM), indomethacin ($10^{-5}\ \text{M}$), propranolol ($10^{-7}\ \text{M}$) or nifedipine ($10^{-6}\ \text{M}$) did not affect the atropine-induced relaxation (Table 1). In addition, removal of endothelium also did not have any significant effect on the responses to atropine (Table 1). Prazosin ($10^{-9}\ \text{M}$) partially inhibited the contraction to noradrenaline ($3 \times 10^{-7}\ \text{M}$) ($51.2 \pm 7.8\%$ of the control) but had no effect on relaxation to atropine ($10^{-7}\ \text{M}$ – $10^{-4}\ \text{M}$) (control: $\text{IC}_{50}\ 1.26 \pm 0.21 \times 10^{-5}\ \text{M}$, maximal relaxation $85.2 \pm 3.0\%$; in the presence of prazosin: $\text{IC}_{50}\ 1.0 \pm 0.20 \times 10^{-5}\ \text{M}$, maximal relaxation $86.0 \pm 3.2\%$, $n = 4$). Prazosin ($10^{-10}\ \text{M}$ – $3 \times 10^{-6}\ \text{M}$) also caused

Figure 1 Inhibitory effects of atropine on contractile responses to noradrenaline (a) and KCl (b) of rabbit aorta. The maximal contractions induced by noradrenaline and KCl were $3.6 \pm 0.23\ \text{g}$ ($n = 5$) and $2.3 \pm 0.10\ \text{g}$ ($n = 3$), respectively, and were expressed as 100%. Each value is the mean (s.e.mean shown by vertical bars) of 3 to 5 experiments. Control (●); atropine at $3 \times 10^{-6}\ \text{M}$ (○); $10^{-5}\ \text{M}$ (▲); $10^{-4}\ \text{M}$ (△).

*Significantly different from respective controls ($P < 0.05$).

Figure 2 Vasorelaxation to atropine in rabbit aorta precontracted with noradrenaline. Tissues were precontracted with noradrenaline (3×10^{-7} M) before cumulative additions of atropine ($\bullet$; 10^{-7} M– 10^{-4} M). In some tissues, theophylline ($\circ$; 10^{-3} M) or M & B 22,948 (Δ ; 10^{-4} M) was added before the noradrenaline treatment. The contraction induced by noradrenaline (3×10^{-7} M) in the absence of phosphodiesterase inhibitors prior to the addition of atropine was 2.5 ± 0.56 g ($n = 5$), 1.20 ± 0.10 g ($n = 5$) in the presence of theophylline and 1.10 ± 0.27 g ($n = 5$) in the presence of M & B 22,948. These were expressed as 100% in the figure. Each value is the mean (s.e.mean shown by vertical bars) of 5 experiments.

*Significantly different from the values before the addition of atropine ($P < 0.05$).

†Significantly different from control based on analysis of variance and Dunnett's test ($P < 0.05$).

Table 1 Effects of various treatments on vasorelaxant action of atropine (10^{-7} M– 10^{-4} M) in rabbit aorta precontracted by noradrenaline (3×10^{-7} M)

	Atropine IC_{50} ($\times 10^{-5}$ M)	Maximal relaxation (%)
Control	1.18 ± 0.38	92.2 ± 2.3
Tetraethyl ammonium (10 mM)	1.78 ± 0.41	91.3 ± 1.8
Indomethacin (10^{-5} M)	0.98 ± 0.21	92.2 ± 1.8
Nifedipine (10^{-6} M)	0.81 ± 0.28	95.0 ± 1.4
Propranolol (10^{-7} M)	1.41 ± 0.31	96.6 ± 1.8
Endothelium-denuded	1.06 ± 0.20	87.6 ± 3.1

IC_{50} values (concentrations of atropine that inhibited 50% of the noradrenaline response) were calculated graphically. In endothelium-denuded tissues, endothelium of the aorta was removed by rubbing the tissues with wet filter paper. Each value is the mean $\pm$ s.e.mean of 5 experiments.

relaxation of the aorta precontracted by noradrenaline (3×10^{-7} M) (IC_{50} $5.96 \pm 0.32 \times 10^{-10}$ M, maximal relaxation 100%, $n = 4$). Pretreatment with atropine (10^{-5} M) partially inhibited the noradrenaline (3×10^{-7} M)-induced contraction ($64.2 \pm 10.9\%$ of the control) but had no effect on relaxation to prazosin (IC_{50} $6.03 \pm 0.29 \times 10^{-10}$ M, maximal relaxation 100%, $n = 4$).

In a Ca^{2+} -free medium containing EGTA (0.01 mM) and nifedipine (10^{-6} M), noradrenaline (3×10^{-7} M) induced a phasic contraction. The subsequent addition of Ca^{2+} (1.2 mM) induced a contractile response, presumably related to the activation of non-voltage operated Ca-channels. Pretreatment with atropine (3×10^{-7} M– 10^{-4} M) inhibited

the residual contraction to noradrenaline in a Ca^{2+} -free medium (Figure 3a) and the subsequent contraction to Ca^{2+} (Figure 3b), both in a concentration-dependent manner. Pretreatment of the aorta with theophylline (10^{-3} M) or forskolin (3×10^{-7} M) also inhibited the residual noradrenaline response (Figure 4a) and the subsequent response to Ca^{2+} (Figure 4b). However, the inhibitory effect of a combined treatment with atropine (10^{-5} M) plus theophylline (10^{-3} M) or atropine (10^{-5} M) plus forskolin (3×10^{-7} M) was much greater than a single treatment with either atropine (10^{-5} M), theophylline (10^{-3} M) or forskolin (3×10^{-7} M) (Figure 4a,b). Similarly, pretreatment of the aorta with prazosin (3×10^{-9} M) inhibited the residual noradrenaline response (Figure 5a) and the subsequent Ca^{2+} -response (Figure 5b). The inhibitory action of a combined treatment with atropine (10^{-5} M) plus prazosin (3×10^{-9} M) was also significantly greater than that of a single treatment with either agent (Figure 5a,b).

Effect of atropine on cyclic AMP, inositol phosphates and [3H]-prazosin binding

Atropine had no significant effect on cyclic AMP levels (control 128.5 ± 6.1 pmol g $^{-1}$ net weight; atropine at 10^{-6} M, 133.2 ± 4.4 ; 10^{-5} M, 124.6 ± 13.0 ; 10^{-4} M, 125.7 ± 11.0 ; $n = 4$).

Noradrenaline (10^{-5} M) in the presence of LiCl (10 mM) caused an increase in the level of [3H]-inositol monophosphate (IP) (Figure 6). Atropine (10^{-6} M– 10^{-4} M) inhibited the increase in the [3H]-IP level, in a concentration-dependent manner (Figure 6). Treatment with prazosin (3×10^{-9} M) or theophylline (10^{-3} M) also inhibited the increase in the [3H]-IP level (Figure 6). In addition, the effect of a combined treatment with atropine (10^{-5} M) plus prazosin (3×10^{-9} M) was significantly greater than that of a single treatment with either agent (Figure 6). Similarly, the inhibitory effect of combined treatment with atropine (10^{-5} M) plus theophylline (10^{-3} M) was much greater than that of a single treatment with either agent (Figure 6).

A possible inhibitory effect of atropine via α -adrenoceptors was investigated with [3H]-prazosin binding in rabbit aorta.

Figure 3 The effect of atropine in Ca^{2+} -free solution on the residual contraction to noradrenaline, and to Ca^{2+} in the presence of noradrenaline in rabbit aorta. Tissues were incubated in Ca^{2+} -free solution containing EGTA (0.01 mM) and nifedipine (10^{-6} M) for 15 min before (a) the addition of noradrenaline (3×10^{-7} M). (b) Ca^{2+} (1.2 mM) was added to the bath 15 min later. Atropine was added to the bath 10 min before the addition of noradrenaline. The contractions induced by (a) noradrenaline and (b) Ca^{2+} were 1.5 ± 0.21 g ($n = 6$) and 2.2 ± 0.27 g ($n = 6$), respectively, in the absence of atropine. Each value is the mean (s.e.mean shown by vertical bars) of 4 to 7 experiments.

*Significantly different from respective controls ($P < 0.05$).

Figure 4 Effects of atropine, theophylline and forskolin on the residual contraction in response to noradrenaline and Ca²⁺ in the presence of noradrenaline in Ca²⁺-free solution. Tissues were incubated in Ca²⁺-free medium containing EGTA (0.01 mM) and nifedipine (10⁻⁶ M) for 15 min before (a) the addition of noradrenaline (3 × 10⁻⁷ M). (b) Ca²⁺ (1.2 mM) was then added to the bath 15 min later. Atropine (Atrop; 10⁻⁵ M), theophylline (Theoph; 10⁻³ M), forskolin (Forsk; 3 × 10⁻⁷ M), atropine (10⁻⁵ M) plus theophylline (10⁻³ M) (Atrop + Theoph), or atropine (10⁻⁵ M) plus forskolin (3 × 10⁻⁷ M) (Atrop + Forsk) was added to the bath 10 min before the addition of noradrenaline. The contractions induced by (a) noradrenaline and (b) Ca²⁺ were 1.5 ± 0.10 g (n = 5) and 2.3 ± 0.23 g (n = 5), respectively. Each value is the mean ± s.e.mean of 4 to 5 experiments.

*Significantly different from respective controls in the absence of inhibitors ($P < 0.05$).

†Significantly different from respective single treatments based on analysis of variance and Dunnett's test ($P < 0.05$).

In order to evaluate this interaction the characteristics of binding of [³H]-prazosin to α -receptor sites in rabbit aorta was established over a concentration-range of 0.1–10 nM [³H]-prazosin. Rosenthal analysis (Rosenthal, 1967) of the saturation isotherm of binding revealed a monophasic plot with a $B_{\max}$ of 28 fmol mg⁻¹ protein and a K_D of 0.44 nM (data not shown). Atropine (10⁻⁸ M–10⁻⁴ M) did not displace specific [³H]-prazosin binding from the α -receptor site. Expressed as % of control [³H]-prazosin binding (0.5 nM), binding varied from 99–109% in the presence of atropine.

Discussion

In the rabbit aorta, atropine inhibited contractile responses to noradrenaline, in a non-competitive manner, but had no effect on the response to KCl. Muscarinic receptors do not appear to be involved because the concentrations of atropine required were substantially higher than those effective at muscarinic receptors, and because other muscarinic antagonists did not produce similar inhibition. In addition, the results indicate that the relaxant action of atropine is not related to K⁺ channels sensitive to TEA, voltage-operated Ca²⁺ channels, prostaglandins or the activation of β -adrenoceptors, since the effect of atropine was not affected by TEA, indomethacin (cyclo-oxygenase inhibitor), nifedipine (inhibitor for voltage-operated Ca²⁺ channels) or propranolol (β -adrenoceptor antagonist). The results also indicate that removal of the endothelium does not affect the relaxation to atropine, suggesting that the production of endothelium-derived relaxing factors is not involved in these responses. The present study also indicates that the relaxation to atropine is not related to interaction with α_1 -adrenoceptors, because the relaxant action of either atropine or prazosin (low concentrations), an α_1 -adrenoceptor antagonist, was not affected by pretreatment with prazosin or atropine, respectively. In addition, potentiation by theophylline, an inhibitor

Figure 5 Effects of atropine and prazosin on the residual response to noradrenaline and on the Ca²⁺ response in the presence of noradrenaline in rabbit aorta. Tissues were incubated in Ca²⁺-free medium containing EGTA (0.01 mM) and nifedipine (10⁻⁶ M) for 15 min before (a) the addition of noradrenaline (3 × 10⁻⁷ M). (b) Ca²⁺ (1.2 mM) was then added to the bath 15 min later. Atropine (Atrop; 10⁻⁵ M), prazosin (Praz; 3 × 10⁻⁹ M), or atropine (10⁻⁵ M) plus prazosin (3 × 10⁻⁹ M) (Atrop + Praz) was added to the bath 10 min before the addition of noradrenaline. The contractions induced by (a) noradrenaline and (b) Ca²⁺ were 1.8 ± 0.21 g (n = 6) and 2.10 ± 0.17 g (n = 6), respectively. Each value is the mean (s.e.mean shown by vertical bars) of 4 to 6 experiments.

*Significantly different from respective controls in the absence of any inhibitor ($P < 0.05$).

†Significantly different from respective single treatments based on analysis of variance and Dunnett's test ($P < 0.05$).

of cyclic AMP-phosphodiesterase, but not with M & B 22,948, an inhibitor of cyclic GMP-phosphodiesterase (Kukovetz *et al.*, 1979) suggests an involvement of cyclic AMP rather than cyclic GMP in the relaxation to atropine.

Agents which interfered with the intracellular mobilization of Ca²⁺ inhibited the residual noradrenaline-induced contraction in a Ca²⁺-free solution in a number of investigations (Weishaar *et al.*, 1983; Hester, 1985; Shibata *et al.*, 1987; 1988). The results in the present study indicated that atropine inhibited the residual noradrenaline response in Ca²⁺-free solution containing EGTA and nifedipine. Atropine also inhibited the Ca²⁺ response in the presence of noradrenaline in Ca²⁺-free solution. The inhibitory effect of atropine on the residual noradrenaline response was much greater than that on the response to Ca²⁺ in the presence of noradrenaline. This may be due to the differing background levels of tension. This agonist-induced contraction is nifedipine-insensitive, so is probably due to the activation of voltage-dependent Ca²⁺-channels (Karaki *et al.*, 1984; Hester, 1985; Shibata *et al.*, 1987; 1989). The present results suggest the possibility that atropine may interfere with intracellular

Figure 6 Effects of atropine, prazosin and theophylline on the level of inositol monophosphate (IP) in rabbit aorta. The tissue level of IP in the presence of noradrenaline (10^{-5} M) was measured in the presence of LiCl (10 mM) as described in the Methods section. In some experiments, tissues were incubated with atropine (Atrop; 10^{-6} M, 10^{-5} M and 10^{-4} M), prazosin (Praz; 3×10^{-9} M), theophylline (Theoph; 10^{-3} M), atropine (10^{-5} M) plus prazosin (3×10^{-9} M) (Atrop + Praz), or atropine (10^{-5} M) plus theophylline (10^{-3} M) (Atrop + Theoph) in addition to noradrenaline (10^{-5} M). The value for control (LiCl) in the absence of the drug was 2.92 ± 0.89 c.p.m. mg^{-1} net weight ($n=15$). Each value is the mean (s.e.mean shown by vertical bars) of 4 to 7 experiments. *Significantly different from the noradrenaline-control in the absence of inhibitors ($P<0.05$).

†Significantly different from respective single treatments based on analysis of variance and Dunnett's test ($P<0.05$).

Ca^{2+} mobilization and also Ca^{2+} -influx through receptor-operated Ca^{2+} channels.

The results also indicated that pretreatment with either theophylline or forskolin, an activator of adenylyl cyclase (Seamon *et al.*, 1981), inhibited both the residual noradrenaline response and the subsequent Ca^{2+} response. The inhibitory effects of combined treatments with atropine plus theophylline and atropine plus forskolin, however, were significantly greater than that of a single treatment with any one of these agents. If atropine was increasing cyclic AMP by inhibiting the activity of cyclic AMP-phosphodiesterase, atropine would not be expected to cause further inhibition of the responses in the presence of theophylline, since the high concentration of theophylline should have completely inhibited the enzyme activity. If atropine increases cyclic AMP by activating adenylyl cyclase at the site which forskolin activates, the effect of atropine in the presence of forskolin should not be different from the effect of single treatment. However, the results suggest that atropine may either activate the enzyme at a different site from forskolin or that the effect of atropine may not be related to activation of

adenylyl cyclase. In fact, in the present study, atropine failed to affect the level of cyclic AMP. These results indicate that the mode of inhibitory action of atropine is not directly related to the absolute level of cyclic AMP. It has been proposed that phosphorylation of phospholipase C- γ by cyclic AMP-dependent protein kinase may alter its interaction with GTP-binding protein and leads to its inhibition (Kim *et al.*, 1989). Alternatively, the GTP-binding protein may be the target of cyclic AMP-dependent protein kinase (Abdel-Latif, 1991). Since atropine is lipophilic, it is conceivable that atropine may also enter the vascular smooth muscle cells and inhibit the activity of phospholipase C or G-protein itself. However, the exact mechanism for the interaction between the effect of atropine and the cyclic AMP level cannot be ascertained at this time. The results further indicated that the inhibitory effect of a combined treatment with atropine and a low concentration of prazosin was significantly greater than that of a single treatment with either agent. These results also suggest that the mechanism of action of atropine is different from that of prazosin.

The hydrolysis of inositol lipids is known to be linked to calcium signalling (Berridge & Irvine, 1984). Activation of α -adrenoceptors increases the metabolism of phosphoinositol 4,5-bisphosphate into two second messengers, inositol trisphosphate (IP_3) and diacylglycerol (Abdel-Latif, 1983; Berridge & Irvine, 1984). IP_3 and diacylglycerol release intracellular calcium and activate protein kinase C, respectively (Berridge & Irvine, 1984; Williamson *et al.*, 1985; Abdel-Latif, 1986). In the present investigation, atropine and prazosin both inhibited increases in inositol monophosphate (IP), a metabolite of IP_3 , in the presence of noradrenaline and lithium. However, the effect of a combined incubation with atropine plus prazosin on the IP level was much greater than that with either agent alone. These results, therefore, indicate that the effect of atropine may be due to inhibition of the metabolism of membrane phosphoinositide and further illustrate the possibility that the mechanism of action of atropine is different from prazosin. This is further supported by the finding that atropine does not affect the binding of [^3H]prazosin.

The results also indicated that theophylline could partially inhibit the accumulation of IP. However, the inhibitory effect of a combined treatment with atropine plus theophylline was much greater than that of a single treatment with either agent. Therefore, the effect of atropine on the phosphoinositide metabolism may also be different from that of theophylline.

In summary, atropine inhibited contraction to noradrenaline in the rabbit aorta, without significantly affecting the response to KCl. Atropine also inhibited the noradrenaline response due to intracellular Ca^{2+} release, and the response due to Ca^{2+} influx through receptor-operated channels. The inhibitory effect of atropine was not apparently due to an interaction with α_1 -adrenoceptors but may reflect inhibition of phosphoinositide metabolism.

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