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Short communication

Is α-adrenoceptor blockade responsible for atropine flush?

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Abstract

Toxic amounts of atropine usually, and therapeutic doses occasionally, dilate cutaneous blood vessels, especially those in the blush area (atropine flush). However, the mechanism of this anomalous vascular response is unknown. We, therefore, investigated this action of atropine not only in functional but also in binding studies with isolated rat aorta and brain, respectively. Endothelium-denuded rat thoracic aortic rings were contracted with norepinephrine, U46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$, thromboxane A_2 receptor agonist) and KCl, and the relaxation in response to atropine was recorded. The norepinephrine-, but not the U46619- or KCl-mediated contraction was relaxed by atropine. Atropine caused a rightward parallel shift of the phenylephrine concentration-contraction curve with a pA_2 value of 6.57 (slope 0.58), but did not affect the concentration-contraction curve for U46619. In rat cerebral cortex homogenates, atropine displaced [^{3}H]prazosin binding with a K_i value of 1.21 μM, while phentolamine and clonidine displaced [^{3}H]prazosin with K_i values of 3.33 nM and 0.19 μM, respectively. These results suggest that even though atropine has low affinity for the α-adrenoceptor, it possesses characteristics similar to those of a competitive ligand for the α-adrenoceptor. Thus, atropine, especially at high concentrations, has direct α-adrenoceptor blocking activity, which may account, at least in part, for atropine flush.

Keywords: Atropine; α-Adrenoceptor; Aorta; Cerebral cortex; (Rat)

1. Introduction

Atropine and related compounds are competitive antagonists of acetylcholine and other muscarinic receptor agonists as they compete with such agonists for a common binding site on the muscarinic receptor (Yamamura and Snyder, 1974; Hulme et al., 1978). Clinically effective doses of atropine are, essentially, without central nervous system effects. However, at toxic doses, atropine causes central nervous system stimulation, followed by depression, which leads to circulatory collapse and respiratory failure after a period of paralysis and coma (Brown, 1990). In the cardiovascular system, toxic amounts of atropine usually dilate cutaneous blood vessels, termed 'atropine flush'. The mechanism underlying the atropine-mediated vasodilation is not clear, although it has been suggested that the dilation is due to a direct action of atropine unrelated to cholinergic blockade (Brown, 1990). Thus, we investigated the mechanism of this anomalous vasodilator action of atropine, using isometric tension as well as receptor binding studies.

2. Materials and methods

2.1. Materials

Phenylephrine HCl (PE), *l*-norepinephrine bitartrate (NE), clonidine HCl and indomethacin were purchased from Sigma Chemical Co. (St. Louis, USA). Phentolamine mesylate and U46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$) were from Bio Mol (USA) and [^{3}H]prazosin (specific activity 78 Ci/mmol) from NEN (USA).

2.2. Tissue preparations

Male Sprague-Dawley rats (12–15 weeks, 250–300 g) were decapitated, and the cerebral cortex and tho-

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racic aorta were removed. In the isometric tension studies, the aorta was cleaned of adhering fat and connective tissue, and ring preparations were prepared as we have previously described (Chang et al., 1993). The endothelium was removed by gently rubbing the intimal surface of the ring with a wooden stick. In the binding studies, cortical tissues were homogenized in 20 volumes of assay buffer (50 mM Tris, 5 mM $MgSO_4$, 1 mM EDTA, 1 mM ascorbic acid, pH 7.7, 25°C), using an Ultraturrax homogenizer (13500 rpm, 15 s × 2). The homogenate was centrifuged 3 times for 15 min at 35000 × g, and the pellet was resuspended each time in 20 volumes of assay buffer. After the final centrifugation, the supernatant was aspirated and pellet was stored at −70°C. The frozen pellet was resuspended in assay buffer just before the binding assay was performed. The final tissue concentration in the binding assay was 5 mg/ml.

2.3. Isometric tension study

Each ring was mounted in a 10 ml water jacketed muscle chamber containing 37°C modified Krebs-Ringer bicarbonate solution which was gassed with 95% O_2-5% CO_2 and had the following composition (mM): NaCl (136.9), KCl (5.4), $MgCl_2$ (1.0), $NaHCO_3$ (23.8), $CaCl_2$ (1.5), glucose (5.5) and EDTA (0.03). High K^+ solution was made by substituting equimolar KCl for 60 mM NaCl in the Krebs-Ringer solution. Tissue was equilibrated at 1 g tension for more than 90 min, with washing at 20 min intervals, prior to drug addition. Isometric tension was recorded on a Grass physiograph (model 7E) using a force displacement transducer (FT-03). To test the relaxant effect of atropine on tone induced by different agonists, plateau contractions in response to either norepinephrine, U46619 or KCl were elicited, followed by cumulative concentrations of atropine. To test the ability of atropine to inhibit agonist-induced contraction, tissues were pretreated (15 min) with atropine, followed by cumulative addition of phenylephrine or U46619. Experiments were performed in the presence of indomethacin (1 μM) to prevent the potential effects of the secondary release of prostanoids.

2.4. [^{3}H]Prazosin binding study

Binding experiments were performed essentially as described (Mohell et al., 1983). In equilibrium binding studies, different concentrations (50 pM to 2 nM) of [^{3}H]prazosin were incubated with membranes in a shaking water bath at 25°C for 30 min. The reaction was stopped with 10 ml ice-cold washing buffer (50 mM Tris, pH 7.7, 25°C), and the incubation mixture was immediately filtered through Whatman GF/C glass microfiber filters under vacuum. Wet filters were placed in mini-vials, to which scintillation cocktail was added, and the vials were shaken for 2 h and then counted. Competition experiments were performed as above, but 200 pM [^{3}H]prazosin and various concentrations of atropine, phentolamine and clonidine were used. Using the iterative nonlinear least-square curve fitting program, data for saturation and competition experiments were analyzed (Lundon Software, Chagrin Falls, OH, USA).

2.5. Statistics

Data are expressed as means ± S.E.M. Differences between two groups were determined by Student's

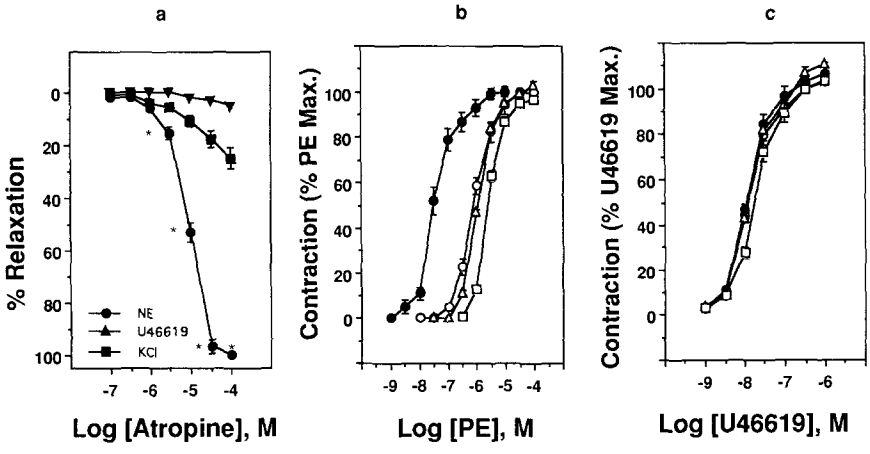

Fig. 1. Concentration-response curves for the effects of atropine on various stimulant-induced contractions (a), and effects of atropine on the concentration-response curves of phenylephrine (PE)-mediated (b) and U46619-mediated contraction (c) in rat thoracic aorta. ●: control, and ○, △, and □: 10, 30 and 100 μM atropine, respectively. Each point represents the mean ± S.E.M. of 3–5 different experiments. $^*P < 0.001$ (norepinephrine, NE vs. U46619 and KCl).

t-test and were considered significantly different if $P < 0.05$. Drug concentrations were expressed as the negative log molar concentration.

3. Results

3.1. Vascular relaxation action of atropine

Atropine concentration dependently relaxed norepinephrine-contracted rat aorta. In contrast, U46619-induced tone did not relax in response to atropine. Atropine concentrations that completely relaxed the norepinephrine-induced tone, relaxed KCl-induced tone by only about 30% (Fig. 1a).

3.2. Inhibitory action of atropine on U46619- and phenylephrine-induced contractions

To test whether the relaxant effect of atropine is specific for norepinephrine, we contracted vessels with the α-adrenoceptor agonist, phenylephrine. Atropine concentration dependently and competitively inhibited phenylephrine-induced contractions (Fig. 1b). When assessed by Schild plot analysis (Arunlakshana and Schild, 1958), atropine showed a pA_2 of 6.57 for α-adrenoceptors in rat thoracic aorta with a slope of 0.58. In contrast, the concentration-contraction curve for U46619 was not affected by atropine (Fig. 1c).

3.3. Effects of atropine, phentolamine and clonidine on [^{3}H]prazosin binding

To test whether the ability of atropine to inhibit α-adrenoceptor-mediated contractions was due to competition at this receptor, we examined the ability of atropine to displace [^{3}H]prazosin binding in the rat brain, a tissue in which α-adrenoceptors are abundant (Glossmann and Hornun, 1980; Greenberg et al., 1976; U'Prichard and Snyder, 1978). Furthermore, in order to compare the relative potency of atropine for α-adrenoceptors, we used known ligands of α-adrenoceptors, namely phentolamine and clonidine. The specific binding of [^{3}H]prazosin, a well characterized selective ligand for α_1-adrenoceptor in vascular smooth muscle and in cerebral cortex (Cohen et al., 1979; Oshita et al., 1991), yielded linear plots from which K_d and B_{max} were derived as 133.5 ± 8.91 pM and 15.15 ± 0.64 fmol/mg, respectively. The effects of atropine, phentolamine and clonidine on [^{3}H]prazosin binding are depicted in Fig. 2. The inhibition constant (K_i) for phentolamine and clonidine was 3.33 ± 0.2 nM and 0.19 ± 0.12 μM, respectively. In contrast, atropine displaced [^{3}H]prazosin binding with a K_i value of 1.21 ± 0.07 μM.

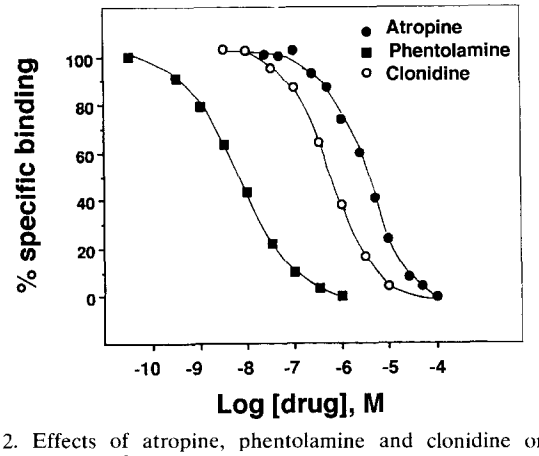

Fig. 2. Effects of atropine, phentolamine and clonidine on the displacement of [^{3}H]prazosin binding in rat cerebral cortex. Each point represents the mean ± S.E.M. of six different experiments.

4. Discussion

The present study demonstrates that atropine, in addition to its well-known interaction at muscarinic receptors (Yamamura and Snyder, 1974; Hulme et al., 1978), interacts at α-adrenoceptors, albeit at much higher concentrations, as indicated by studies of isometric contraction in isolated rat aorta, and by binding studies in rat cerebral cortex. Since atropine inhibited contractions produced by an α-adrenoceptor agonist, but not by U46619 or KCl, the relaxant effects of atropine appear to the selective for α-adrenoceptor-mediated contraction. Similar results were obtained in mouse thoracic aorta (data not shown), suggesting that the vasodilator action of atropine is not species-dependent. Consistent with the selective inhibition of α-adrenoceptor-mediated contraction is the displacement of [^{3}H]prazosin from rat cerebral cortex by atropine.

In conclusion, we demonstrated that atropine has considerable affinity for α-adrenoceptors in rat cerebral cortex and has the ability to antagonize α-adrenoceptor-mediated contractions in rat thoracic aorta. These results indicate that the direct α-adrenoceptor blocking activity of atropine may account, at least in part, for atropine flush. These results also demonstrate that caution must be used in the interpretation of data in which atropine is used with an α-adrenoceptor agonist, especially at higher atropine concentrations.

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