J. Pharm. Pharmacol. 1998, 50: 317–322
Received October 1, 1997
Accepted November 26, 1997

The 5-HT and α-Adrenoceptor Antagonist Effect of Four Benzylisoquinoline Alkaloids on Rat Aorta

M. CATRET, M. D. IVORRA, M. P. D'OCÓN AND E. ANSELMI

Laboratorio de Farmacognosia y Farmacodinamia, Departamento de Farmacología, Facultad de Farmacia, Universidad de Valencia, Avda. Vicent Andrés Estellés s/n 46100, Burjasot, Valencia, Spain

Abstract

The action of four benzylisoquinoline alkaloids (two aporphines—glaucine and apomorphine, a benzylisoquinoline—papaverine and a bisbenzyltetrahydroisoquinoline—antioquine) on 5-HT-induced contraction in rat thoracic aorta has been examined and compared with that of the control drugs: ketanserin, nifedipine, prazosin and phentolamine. The relaxant action on 5-HT-induced contraction was contrasted with that on the contraction induced by noradrenaline and KCl.

The results obtained with control drugs show that ketanserin has clear selectivity for 5-HT receptors, whereas prazosin and phentolamine have high selectivity for the α_1-adrenoceptor and nifedipine seems to have a more potent effect on KCl-induced contraction than on that induced by 5-HT or noradrenaline. The contraction evoked by 5-HT (10 μM) was inhibited in a concentration-dependent manner by all the alkaloids. The order of potency was: papaverine = glaucine > apomorphine > antioquine. Papaverine had a non-specific relaxant action on 5-HT-, noradrenaline- and KCl-induced contraction, antioquine had a weak relaxant action on the agonist assays, and glaucine and apomorphine inhibited noradrenaline- and 5-HT-induced contraction more potently than they inhibited the K^+-depolarized response.

These results indicate that the aporphines assayed, *S*-glaucine and *R*-aporphine, had selective action against agonist (noradrenaline or 5-HT)-induced contraction rather than against KCl-depolarization of rat aorta. In contrast papaverine, a benzylisoquinoline alkaloid, relaxes all agents used non-selectively as could be expected from the lack of specificity that characterizes this alkaloid.

In previous work we have shown that a series of benzylisoquinoline derivative alkaloids structurally related to papaverine (glaucine, laudanosine, antioquine and boldine) have a relaxant effect on the vascular smooth muscle that is related to their capacity to inhibit Ca^{2+} influx through voltage-operated Ca^{2+} channels, act as α_1-adrenoceptor antagonists and inhibit all or some of the different forms of cyclic nucleotide phosphodiesterases isolated from the aorta (Anselmi et al 1992, 1994; Ivorra et al 1992, 1993a, b; Chuliá et al 1994).

Because of the large extent of cross-reactivity between compounds interacting at α-adrenoceptors and 5-HT receptors (Apperley et al 1976; Black et al 1981; Purdy et al 1987), in this study we have examined the action of some of these compounds that are representative of the different structural groups. We selected two aporphine alkaloids – glaucine and apomorphine (Figure 1), a benzylisoquinoline alkaloid – papaverine and a bisbenzyltetrahydroisoquinoline alkaloid – antioquine, studied their action on 5-HT-induced contraction and have compared the effects of these compounds with ketanserin, a 5-HT_2 antagonist, nifedipine, an established calcium antagonist, prazosin, a selective α_1-adrenoceptor antagonist, and phentolamine, a non-selective α-adrenoceptor antagonist.

Correspondence: E. Anselmi, Laboratorio de Farmacognosia y Farmacodinamia, Departamento de Farmacología, Facultad de Farmacia, Universidad de Valencia, Avda. Vicent Andrés Estellés s/n 46100, Burjasot, Valencia, Spain.

Materials and Methods

Thoracic aortic rings (denuded of endothelium) of male Wistar rats, 200–220 g, were prepared and

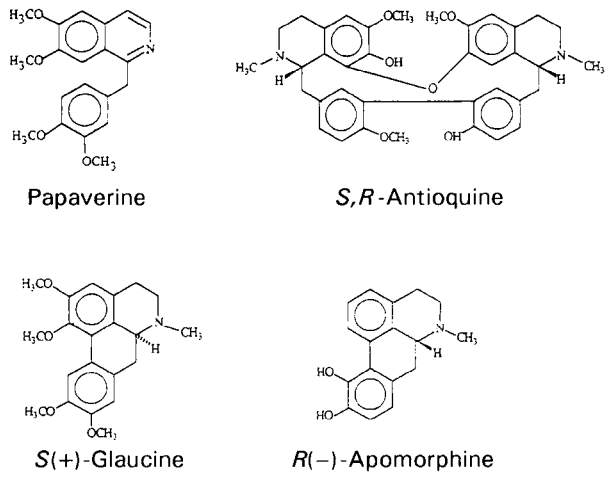

Figure 1. The chemical structures of the alkaloids investigated.

mounted. Each preparation was suspended in a 10-mL organ bath containing Krebs-bicarbonate solution maintained at 37°C and oxygenated with 95% O_2–5% CO_2. An initial load of 1 g was applied to each preparation and maintained throughout a 75–90 min equilibration period before addition of agonist. Tension was recorded isometrically on a Phillips recorder (PM 8222) coupled to a Hewlett-Packard amplifier (8805D) via force-displacement transducers (Gould Statham UC2).

The absence of relaxant response after addition of 10^{-4} M acetylcholine to preparations previously contracted with noradrenaline (1 μM) indicated the absence of a functional endothelium (Furchgott & Zawadzki 1980).

The experiments were performed in Krebs-bicarbonate solution to determine the extent to which the compounds relaxed rat aorta pre-contracted by 5-HT (10 μM), noradrenaline (1 μM) or KCl (60 mM). Contractions were expressed in mg. Concentration–response curves of relaxation were obtained by addition of cumulative concentrations of the compounds after sustained contractions with 5-HT, noradrenaline or KCl. Relaxation was expressed as a percentage of the maximum tension obtained after addition of the highest dose of the compounds. Concentration–response curves were analysed by non-linear regression using a computerized iterative procedure (GraphPad Prism 2.0).

Drugs and solutions

L-Noradrenaline L-tartrate and acetylcholine chloride were from Merck, glaucine, nifedipine, prazosin, (+)-*cis*-diltiazem, apomorphine, 5-HT, papaverine and ketanserin were from Sigma, and phentolamine methanesulphonate was from Ciba-Geigy. Antioquine was isolated from *Pseudoxandra sclerocarpa* (Annonaceae) by the method of Cortes et al (1985).

The composition of Krebs-bicarbonate solution was (mM): NaCl 118, KCl 4·75, $CaCl_2$ 1·8, $MgCl_2$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25 and glucose 11.

Nifedipine was dissolved in ethanol (10^{-2} M) and then diluted further with distilled water. The other drugs were dissolved in distilled water. All solutions were prepared daily and the pH was adjusted to 7·0.

Statistical analysis

When analysis of variance showed significant differences ($P < 0.05$), the results were further analysed by the Student–Newman Keuls test. P values < 0.05 were considered to be indicative of significance.

Results

Effect of alkaloids on 5-HT-induced contraction of rat aorta

5-HT at 10 μM induced sustained contraction of isolated rat aortic rings. The magnitude of this contractile response was 646·19 ± 26·6 mg (n = 7); this dose of 5-HT proved to be that inducing the maximum contraction in rat aorta (Noguera & D'Ocón 1993).

All the alkaloids assayed induced concentration–dependent relaxation in aorta pre-contracted with 5-HT (Figure 2A, Table 1). There was no significant difference between the relaxant potency of papaverine ($10^{-6} - 3 \times 10^{-4}$ M) and glaucine ($10^{-7} - 10^{-4}$ M). The values of IC50 (the concentration inducing half the maximum effect) and E_{max} (the maximum effect) for papaverine were 1·94 ± 0·45 μM and 100% (n = 6), respectively; for glaucine they were 3·18 ± 0·83 μM and 93·6 ± 5·1% (n = 7). Apomorphine ($10^{-6} - 3 \times 10^{-4}$ M) also relaxed 5-HT contraction with IC50 and E_{max} values of 12·7 ± 2·6 μM and 95·3 ± 2·5% (n = 6), respectively. Finally antioquine ($10^{-6} - 3 \times 10^{-4}$ M) was clearly the least potent of the alkaloids; its maximum relaxant effect was 60·8 ± 2·4% and its IC50 value 223·0 ± 43·0 μM (n = 7). After washing out of the alkaloid, no recuperation of the contractile response of 5-HT was observed.

The rank order of potency of these alkaloids on 5-HT-induced contraction was papaverine = glaucine > apomorphine > antioquine.

Effects of the control drugs on 5-HT-, noradrenaline- and KCl-induced contraction of rat aorta

The effect of cumulative concentrations of the different compounds on aorta pre-contracted with 5-HT was also investigated (Figure 2B, Table 1). Ketanserin ($10^{-10} - 10^{-8}$ M) was the most potent

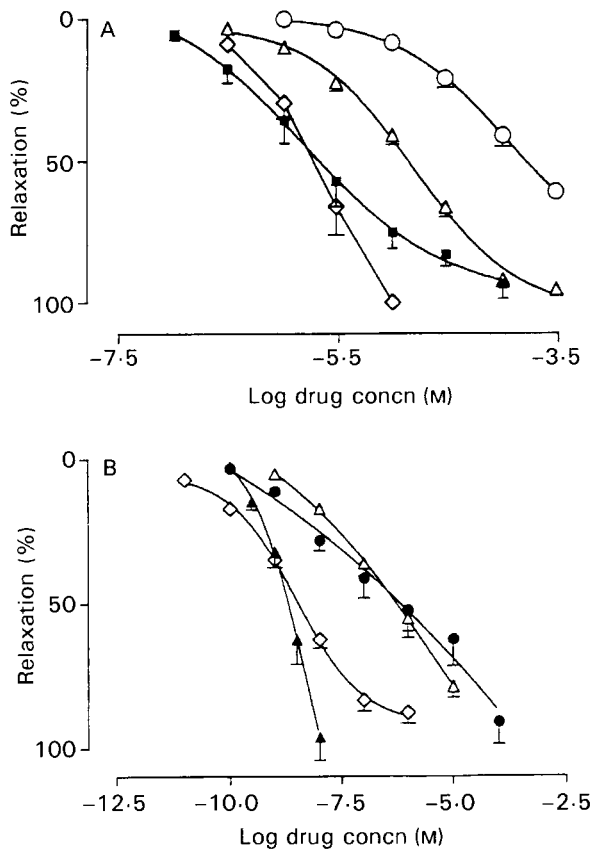

Figure 2. Dose–response curves for the relaxation obtained after addition of the different compounds to rat aortae previously contracted by treatment with 10 μM 5-HT. A ■, Glaucine; △, apomorphine; ○, antioquine; ◇, papaverine. B ▲, Ketanserin; ◇, nifedipine; △, phentolamine; ●, prazosin. Each point is the mean of results from n experiments; the vertical bars represent the s.e.m.

relaxant (IC50 $1{\cdot}9 \pm 0{\cdot}4 \times 10^{-3}$ μM; E_{max} 96·0 ± 8·2%; n = 5). The calcium antagonist nifedipine induced concentration-dependent relaxation with IC50 and E_{max} values of $7{\cdot}05 \pm 1{\cdot}6 \times 10^{-3}$ μM and 87·7 ± 3·7% (n = 7), respectively.

The two α-adrenoceptor antagonists also relaxed 5-HT-induced contraction; phentolamine (10^{-11} – 10^{-5} M) was more potent than prazosin (10^{-13} – 10^{-7} M)—the IC50 and E_{max} for phentolamine were 0·35 ± 0·07 μM and 78·8 ± 3·7% (n = 7), respectively, whereas those for prazosin were 2·06 ± 1·16 μM and 91·0 ± 7·6% (n = 3). After product washout no recuperation of the contractile response of 5-HT was observed except with ketanserin and phentolamine. The rank order of potency of these compounds on 5-HT-induced contraction was ketanserin > nifedipine > phentolamine > prazosin.

Noradrenaline at 1 μM induced sustained contraction (348·8 ± 31·0 mg, n = 9) in rat isolated thoracic aorta incubated in Krebs-bicarbonate solution. Addition of cumulative doses of ketanserin, nifedipine, phentolamine and prazosin induced concentration-dependent relaxation (Figure 3); the IC50 values are summarized in Table 1. The rank order of potency of these compounds on noradrenaline-induced contraction was prazosin > nifedipine > phentolamine = ketanserin.

Another series of experiments was performed in Krebs-bicarbonate solution to determine the blocking effect of each product on Ca^{2+}-entry through potential-operated Ca^{2+}-channels. The contractile response of rat aorta was elicited by depolarizing solution (KCl 60 mM); its magnitude was 225·2 ± 18·5 (n = 18). All products tested induced concentration-dependent relaxation (Figure 3); the IC50 values are summarized in Table 1.

Discussion

In rat aorta the activation of the 5-HT_{2A} receptor by addition of 5-HT induces a biphasic contraction (Sufka et al 1990). An initial transient contraction

Table 1. Parameters of the concentration-response curves for relaxation of 5-HT- and noradrenaline-induced contraction and of KCl-depolarized rat aorta by addition of cumulative concentrations of the compounds investigated.

Compound	5-HT IC50 (μM)	Noradrenaline IC50 (μM)	KCl IC50 (μM)	$IC50_{(5\text{-HT})}/IC50_{(noradrenaline)}$	$IC50_{(5\text{-HT})}/IC50_{(KCl)}$
Papaverine	1·94 ± 0·45 (n = 6)	3·65 ± 0·87 (n = 8)†	4·2 ± 1·1 (n = 4)†	0·53	0·46
Antioquine	223·0 ± 43·0 (n = 5)	> 300 (n = 10)‡	133 ± 24·0 (n = 6)‡	< 0·7	1·68
Glaucine	3·18 ± 0·83 (n = 7)*,**	0·89 ± 0·15 (n = 6)**	41·6 ± 4·3 (n = 11)†	3·57	0·076
Apomorphine	12·7 ± 2·6 (n = 5)**	14·4 ± 4·1 (n = 6)§	71·3 ± 3·3 (n = 5)§	0·88	0·18
Ketanserin	$1{\cdot}9 \pm 0{\cdot}4 \times 10^{-3}$ (n = 5)*,**	0·21 ± 0·05 (n = 5)**	8·7 ± 2·3 (n = 3)	$9{\cdot}04 \times 10^{-3}$	$2{\cdot}18 \times 10^{-4}$
Nifedipine	$7{\cdot}05 \pm 1{\cdot}6 \times 10^{-3}$ (n = 7)**	$11{\cdot}0 \pm 2{\cdot}4 \times 10^{-3}$ (n = 5)†,**	$0{\cdot}75 \pm 0{\cdot}15 \times 10^{-3}$ (n = 5)†	0·6	9·4
Phentolamine	0·35 ± 0·07 (n = 6)*,**	0·13 ± 0·03 (n = 7)**	6·7 ± 0·9 (n = 4)	2·69	0·05
Prazosin	2·06 ± 1·16 (n = 3)*,**	$0{\cdot}22 \pm 0{\cdot}04 \times 10^{-3}$ (n = 5)‡,**	48·0 ± 15·1 (n = 4)†	$9{\cdot}23 \times 10^{3}$	0·04

Values are means ± s.e.m. *$P < 0{\cdot}05$ significantly different from result for noradrenaline. **$P < 0{\cdot}05$, significantly different from result for KCl. †Ivorra et al (1992). ‡ Ivorra et al (1993b). §Ivorra et al (1993a).

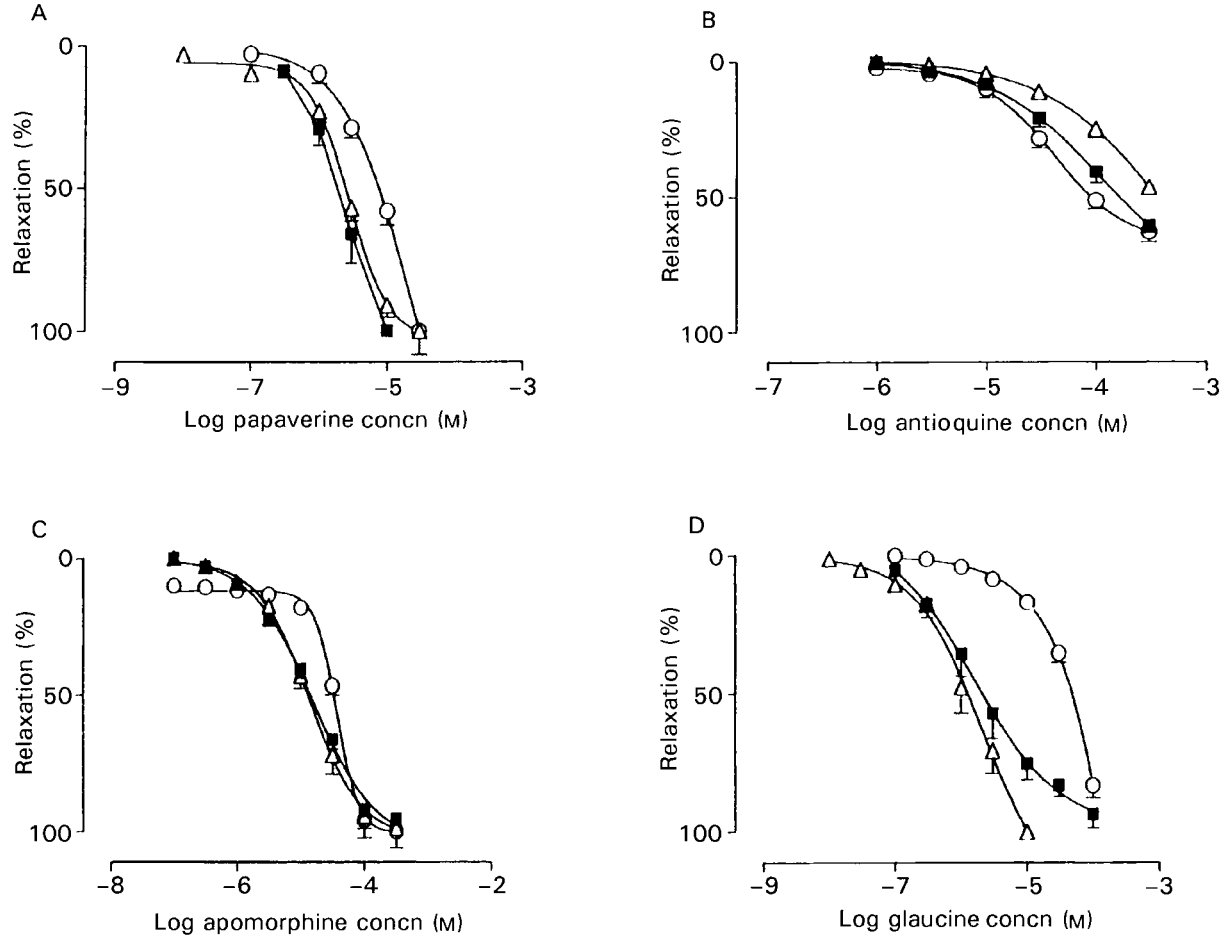

Figure 3. Dose–response curves for the relaxation of ■, 5-HT- (10 μM) and △, noradrenaline- (1 μM) induced contraction and ○, KCl- (60 mM) depolarized rat aorta by: A, papaverine ($10^{-8} - 5 \times 10^{-5}$ M; B, antioquine ($10^{-6} - 3 \times 10^{-4}$ M); C, apomorphine ($10^{-6} - 3 \times 10^{-4}$ M); and D, glaucine ($10^{-7} - 10^{-4}$ M). Each point is the mean of results from n experiments; the vertical bars represent the s.e.m.

produced by IP_3-mediated release of Ca^{2+} from intracellular stores (via activation of IP_3-specific receptors) was followed by a slow sustained contraction caused by Ca^{2+} influx through receptor-operated Ca^{2+} channels (Karaki & Weiss 1988; Van Breeman & Saida 1989).

In the current work the relaxant effect of four alkaloids on 5-HT contraction has been examined. Two of the four alkaloids, glaucine and apomorphine, are apomorphines whereas the others were a benzylisoquinoline (papaverine) and a bisbenzyltetrahydroisoquinoline (antioquine). The results obtained with these were compared with those obtained with several other compounds: ketanserin, a competitive antagonist of the $5\text{-}HT_2$ receptor, nifedipine, a Ca^{2+} antagonist, prazosin, a selective α_1-adrenoceptor antagonist, and phentolamine, a non-selective α-adrenoceptor antagonist. We also contrasted the relaxant action of these compounds on 5-HT-induced contraction with their relaxant action on noradrenaline- and KCl-induced contraction reported in earlier studies (Ivorra et al 1992, 1993 a, b; Anselmi et al 1994).

The results obtained with the control drugs showed ketanserin to have greater selectivity for 5-HT-induced contraction (Figure 4A; Table 1). The $IC50_{(5\text{-}HT)}/IC50_{(noradrenaline)}$ ratio was $9{\cdot}04 \times 10^{-3}$, and the $IC50_{(5\text{-}HT)}/IC50_{(KCl)}$ ratio was $2{\cdot}18 \times 10^{-4}$, the order of this antagonist potency was 5-HT > noradrenaline > KCl. Both α-adrenoceptor antagonists prazosin and phentolamine relaxed 5-HT-induced contraction but were more potent on noradrenaline-induced contraction (Figure 4B, C; Table 1); as expected, prazosin and phentolamine were more selective for the α_1-adrenoceptor; the rank order of potency of both compounds was noradrenaline > 5-HT > KCl. Our results show that ketanserin has greater antagonistic potency than prazosin on 5-HT-induced contraction and means that in rat aorta 5-HT activated the $5\text{-}HT_2$ receptor to a greater extent than it activated the α_1-adrenoceptor. Finally, the Ca^{2+}-antagonist

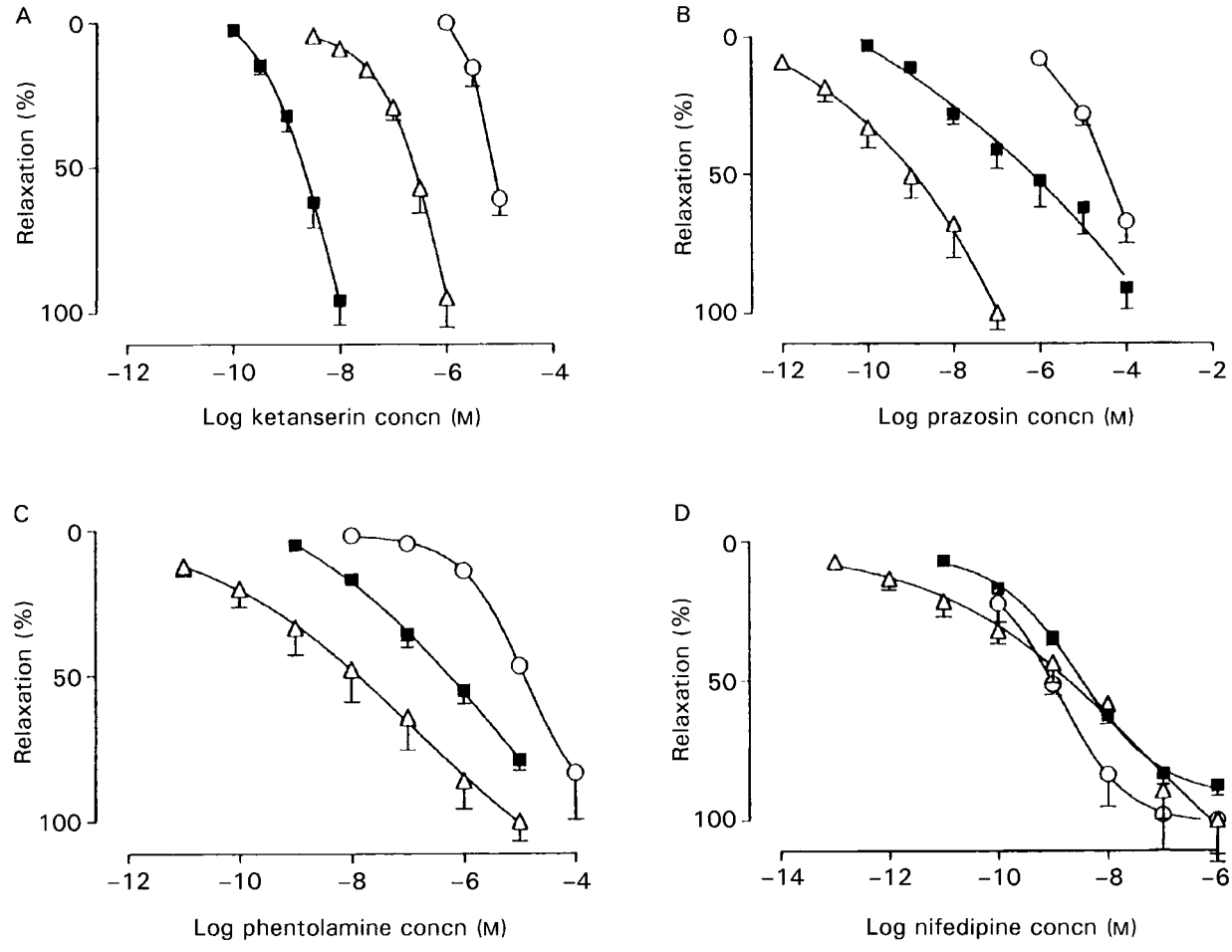

Figure 4. Dose–response curves for the relaxation of ■, 5-HT- (10 μM) and △, noradrenaline- (1 μM) induced contraction and ○, KCl- (60 mM) depolarized rat aorta by: A, ketanserin (10^{-10} – 10^{-8} M); B, prazosin (10^{-13} – 10^{-7} M); C, phentolamine (10^{-11} – 10^{-5}M); and D, nifedipine (10^{-11} – 10^{-6} M). Each point is the mean of results from n experiments; the vertical bars represent the s.e.m.

nifedipine seemed to have more potent effect on KCl-induced contraction than on that induced by 5-HT or noradrenaline (Figure 4D; Table 1). The rank order of potency was KCl > 5-HT = noradrenaline. All the alkaloids relaxed 5-HT-contraction; the rank order of potency was papaverine = glaucine > apomorphine > antioquine (Figure 2A). Comparison of the relaxation induced by these compounds on 5-HT-induced contraction with that induced on noradrenaline- and KCl-induced contraction in previous work (Ivorra et al 1992, 1993a, b) enabled the calculation of the ratios $IC50_{(5\text{-HT})}/IC50_{(noradrenaline)}$ and $IC50_{(5\text{-HT})}/IC50_{(KCl)}$ which give information on selectivity (Table 1) and suggest that papaverine has a non-specific relaxant action on 5-HT-, noradrenaline- and KCl-induced contraction (Figure 3A). These results are in agreement with the well-known spasmolytic behavior of this alkaloid, action which is ascribed to the inhibition of phosphodiesterases (Lugnier et al 1972; Bolton 1979; Cumiskey & Feigenson 1983), the subsequent increase in cyclic AMP and cyclic GMP levels, and modification of intracellular Ca^{2+} distribution. Furthermore, there is evidence that it might also antagonize Ca^{2+} influx (Schneider et al 1975; Reinhardt et al 1977) and act on α-adrenoceptors (Ivorra et al 1992). The results obtained with the bisbenzyltetrahydroisoquinoline alkaloid antioquine show that this compound has a weak relaxant action on 5-HT-induced contraction; similar action has been observed against the agonists noradrenaline and KCl (Ivorra et al 1992) (Figure 3B). The rank order of potency is KCl > 5-HT > noradrenaline. Finally, the results obtained with the aporphine alkaloids show that glaucine and apomorphine induced concentration-dependent inhibition of 5-HT-, noradrenaline- and KCl-induced contraction. Moreover, both aporphines inhibit noradrenaline- and 5-HT-induced contraction more potently than they do K^+-depolarized contraction. These results indicate that the aporphines *S*-glaucine and *R*-apo-

morphine have selectivity of action toward agonist (noradrenaline or 5-HT)-induced contraction compared with that against KCl-depolarization of rat aorta (Figure 3C, D). In contrast papaverine, a benzylisoquinoline alkaloid, relaxes non-selectively; this is to be expected from the lack of specificity that characterize this alkaloid.

It is interesting to note that in previous binding studies Ivorra et al (1992, 1993a, b) demonstrated the capacity of these alkaloids to interact with α_1-adrenoceptors and with benzothiazepine-binding sites, and Orallo et al (1995) observed that in isolated intact rat aorta glaucine competitively inhibited the contractions induced by noradrenaline and non-competitively inhibited those induced by 5-HT. The results obtained with antioquine show a small relaxant action on 5-HT contractile response; this low potency was also observed in a previous study (Ivorra et al 1993b) in which antioquine exhibited a weak relaxant potency on noradrenaline and KCl contractile responses. However, in binding assays this alkaloid completely inhibits binding of [^{3}H]prazosin and [^{3}H]diltiazem to cortical membranes. The discrepancy between the functional and binding results might be because the development of sustained contraction at the highest concentration assayed (300 μM) in the functional studies could prevent the relaxant action of higher doses of the compound.

In summary, these data show that the alkaloids studied can relax smooth muscle cells in rat aorta previously contracted by 5-HT. However the results suggest the possibility of a 5-HT antagonist effect in addition to the α_1-adrenoceptor antagonist properties demonstrated on functional and binding assays. This antagonistic profile might be useful in finding possible clinical applications against several pathophysiological consequences of the co-activation of α_1-adrenoceptors and 5-HT$_2$ receptors, for example essential hypertension, coronary vasospasm and Raynaud's syndrome.

Acknowledgements

This work was supported by Grant no. SAF 95-0538 from the Spanish Commision Interministerial de Ciencia y Tecnología. The authors thank Professor D. Cortes for the donation of antioquine.

References

Anselmi, E., Fayos, G., Blasco, R., Candenas, L., Cortes, D., D'Ocón, P. (1992) Selective inhibition of calcium entry induced by benzylisoquinolines in rat smooth muscle. J. Pharm. Pharmacol. 44: 337–343

Anselmi, E., Gómez-Lobo, M. D., Blázquez, M. A., Zafra-Polo, M. C., D'Ocón, M. P. (1994) Influence of the absolute configuration on the vascular effects of tetrandrine and isotetrandrine in rat aorta. Pharmazie 49: 440–443

Apperley, E., Humphrey, P. P. A., Levy, G. P. (1976) Receptors for 5-hydroxytryptamine and noradrenaline in rabbit isolated ear artery and aorta. Br. J. Pharmacol. 58: 211–221

Black, J. L., French, R. J., Mylecharane, E. J. (1981) Receptor mechanisms for 5-hydroxytryptamine in rabbit arteries. Br. J. Pharmacol. 74: 619–626

Bolton, T. B. (1979) Mechanism of action of transmitters and other substances on smooth muscle. Physiol. Rev. 85: 606–719

Chuliá, S., Ivorra, M. D., Lugnier, C., Vila, E., Noguera, M. A., D'Ocón, P. (1994) Mechanism of the cardiovascular activity of laudanosine: comparison with papaverine and other benzylisoquinolines. Br. J. Pharmacol. 113: 1377–1385

Cortes, D., Sáez, J., Hocquemilles, R., Cavé, A. (1985) Alcaloides des annonacées: alcaloides du *Pseudoxandra off. lucida*: etude de l'antioquine et de ses derivés. J. Nat. Prod. 48: 76–85

Cumiskey, W. R., Feigenson, M. E. (1983) Spasmolytic activity of cinnamedrine and papaverine in isolated rat uterine muscle. Arch. Int. Pharmacodyn. Ther. 263: 113–119

Furchgott, R. F., Zawadzki, J. V. (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288: 373–376

Ivorra, M. D., Lugnier, C., Schott, C., Catret, M., Noguera, M. A., Anselmi, E., D'Ocón, P. (1992) Multiple actions of glaucine on cyclic nucleotide phosphodiesterases, α_1-adrenoceptor and benzothiazepine binding site at the calcium channel. Br. J. Pharmacol. 106: 387–394

Ivorra, M. D., Chuliá, S., Lugnier, C., D'Ocón, M. P. (1993a) Selective action of two aporphines at α_1-adrenoceptors and potential-operated Ca^{2+} channels. Eur. J. Pharmacol. 231: 165–174

Ivorra, M. D., Lugnier, C., Catret, M., Anselmi, E., Cortes, D., D'Ocón, P. (1993b) Investigations of the dual contractile/relaxant properties shown by antioquine in rat aorta. Br. J. Pharmacol. 109: 502–509

Karaki, H., Weiss, G. B. (1988) Calcium release in smooth muscle. Life Sci. 42: 111–122

Lugnier, C., Bertrand, Y., Stoclet, J. C. (1972) Cyclic nucleotide phosphodiesterase inhibition and vascular smooth muscle relaxation. Eur. J. Pharmacol. 19: 134–136

Noguera, M. A., D'Ocón, M. P. (1993) Evidence that depletion of internal calcium stores sensitive to noradrenaline elicits a contractile response dependent on extracellular calcium in rat aorta. Br. J. Pharmacol. 110: 861–867

Orallo, F., Fernández, A., Campos-Toimil, M., Calleja, J. A. (1995) Study of the in vivo and in vitro cardiovascular effects of (+)-glaucine and *N*-carbothoxysecoglaucine in rats. Br. J. Pharmacol. 114: 1419–1427

Purdy, R. E., Murray, D. L., Stupecky, G. L. (1987) Receptors for 5-hydroxytryptamine in rabbit blood vessels: activation of alpha adrenoceptors in rabbit thoracic aorta. J. Pharmacol. Exp. Ther. 240: 535–541

Reinhardt, D., Roggenbach, W., Schmidt, U., Schumann, H. J. (1977) Effects of papaverine on the frequency-force relationship in guinea-pig left atria. Eur. J. Pharmacol. 41: 123–126

Schneider, J. A., Brooker, G., Sperelakis, N. (1975) Papaverine blockade of an inward Ca^{2+} current in guinea-pig heart. J. Mol. Cell. Cardiol. 7: 867–871

Sufka, K. J., Stratton, D. B., Giordano, J. (1990) Regional differences in serotonergic contractile sensitivity mediated by 5-HT$_2$ receptors in rat aorta. Artery 18: 47–53

Van Breemen, C., Saida, K. (1989) Cellular mechanisms regulating $[Ca^{2+}]_i$ in smooth muscle. Annu. Rev. Physiol. 51: 315–329