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The effect of S-(+)-boldine on the α_1-adrenoceptor of the guinea-pig aorta

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1 The cardiovascular activity of S-(+)-boldine, an aporphine alkaloid structurally related to papaverine, was determined. The work includes functional studies on guinea-pig isolated aorta contracted with noradrenaline, caffeine, KCl or Ca^{2+}, and on guinea-pig trachea contracted with acetylcholine or histamine.

2 S-(+)-boldine inhibited in a concentration-dependent manner the contractile reponse evoked by noradrenaline (10 μM) in guinea-pig aorta ($IC_{50} = 1.4 \pm 0.2$ μM) while the KCl depolarizing solution (60 mM)- or the Ca^{2+} (1 mM)-induced contractions were only partially affected by boldine up to 300 μM. In contrast, papaverine relaxed noradrenaline (NA), KCl or Ca^{2+} induced contractions showing similar IC_{50} values in all cases. S-(+)-boldine had a greater potency on the contraction elicited by NA whereas papaverine acted in a non-selective manner.

3 S-(+)-boldine was found to be an α_1-adrenoceptor blocking agent in guinea-pig aorta as revealed by its competitive antagonism of noradrenaline-induced vasoconstriction ($pA_2 = 5.64 \pm 0.08$), and its potency was compared with that of prazosin ($pA_2 = 8.56 \pm 0.24$), a known potent α_1-adrenoceptor antagonist. In contrast, papaverine caused rightward shifts of the NA concentration-response curves with depression of maximal response indicating that it acts as a non-competitive antagonist.

4 Contraction of guinea-pig aorta induced by caffeine (60 mM) in a Ca^{2+}-containing Krebs solution was not affected by a 60 min incubation period with different doses of S-(+)-boldine (1-300 μM). Papaverine inhibited partially this caffeine-induced contraction at the maximal dose used (100 μM).

5 Inositol phosphates formation induced by noradrenaline (10 μM) in guinea-pig thoracic aorta was inhibited by S-(+)-boldine (30 μM) but not by papaverine (10 μM).

6 Contractions of guinea-pig trachea caused by acetylcholine (100 μM) or histamine (10 μM) were not modified by S-(+)-boldine (0.1-100 μM).

7 These results provide evidence that S-(+)-boldine, an aporphine alkaloid, has interesting properties as an α_1-adrenoceptor blocker in vascular smooth muscle, and acts as a competitive antagonist of the α_1-adrenoceptor present in the guinea pig aorta.

Keywords: α_1-adrenoceptor antagonist; S-(+)-boldine; papaverine; prazosin; guinea-pig aorta; inositol phosphates accumulation

Introduction

α-Adrenoceptors are involved in a great variety of physiological processes including regulation of blood pressure. Although the role of α_1-adrenoceptors in hypertensive disease remains unclear, their blockade by appropriate antagonistic drugs is effective in lowering blood pressure, particularly with selective α_1-adrenoceptor antagonists such as prazosin (Cavero & Roach, 1980). Recently, a new group of compounds with the benzylisoquinoline structure has been shown to have α_1-adrenoceptor antagonistic properties in vascular smooth muscle. These compounds are glaucine (Ivorra *et al.*, 1992), apomorphine (Ivorra *et al.*, 1993a), dicentrine (Teng *et al.*, 1991), laudanosine (Chuliá *et al.*, 1994), antioquine (Ivorra *et al.*, 1993b), discretamine (Ko *et al.*, 1993; 1994) and berberine (Bova *et al.*, 1992).

In a previous study, in rat cerebral cortex, we have shown that S-(+)-boldine, an aporphine alkaloid related to papaverine (Figure 1), is able to inhibit [^{3}H]-prazosin and [^{3}H]-(+)-*cis*-diltiazem binding without showing any effect at the dihydropyridine binding site. S-(+)-boldine showed a higher affinity for the α_1-adrenoceptor binding site than for the benzothiazepine receptor site. S-(+)-boldine also relaxed the rat aorta previously contracted by addition of KCl or noradrenaline (Ivorra *et al.*, 1993a).

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The aim of the present work was, firstly, to investigate the antagonist activity of S-(+)-boldine on the α_1-adrenoceptor present in the guinea-pig aorta by use of functional experiments and, secondly, to study the influence of S-(+)-boldine on the phosphoinositide (PI) pathway in this tissue by direct determination of total [^{3}H]-inositol phosphates accumulation, since activation of α_1-adrenoceptors in guinea-pig aorta implies activation of the PI pathway (Jenkin *et al.*, 1991). The relaxant activity of S-(+)-boldine was compared with that of papaverine.

Methods

Contraction studies in guinea-pig isolated thoracic aortic rings

Male guinea-pigs weighing 250-350 g were killed by cervical dislocation. The thoracic aorta was removed and cut into rings. The preparations were mounted isometrically and subjected to an initial tension of 1.0 g in Krebs-Henseleit buffer (KH) at 37°C bubbled with a mixture of 95% O_2 and 5% CO_2. The composition of the KH was (mM): NaCl 119, KCl 5.4, $CaCl_2 \cdot 2H_2O$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11. The Ca^{2+}-free solution had the same composition except that $CaCl_2$ was omitted and EDTA (1 mM) was

added. The depolarizing solution (KCl 60 mM) was obtained by equimolar substitution of NaCl for KCl. Tensions were measured with Pioden strain gauges (UF 1) connected with Celaster AC-261 amplifiers, and were displayed on Linseis L65514 recorders.

Aortae were equilibrated in the medium for 90 min with six changes of KH before specific experimental protocols were initiated. Following a period of equilibration, the resting tension was between 0.35–0.65 g.

In all experiments, the endothelium was removed by gently rubbing the luminal surface; the absence of acetylcholine (1–100 μM)-induced relaxation in preparations previously contracted with noradrenaline (10 μM) was taken as an indicator of the absence of a functional endothelium (Furchgott & Zawadzki, 1980).

The aortic rings were contracted to submaximal tension with either noradrenaline (NA, 10 μM), KCl (60 mM) or Ca^{2+} (1 mM). Concentration-relaxation curves were obtained by addition of cumulative doses of *S*-(+)-boldine (0.01–300 μM) or papaverine (0.01–100 μM) to the precontracted preparations. Maximal relaxation was obtained by addition to the bath of sodium nitroprusside dihydrate (1 μM). Contractions in KH were calculated in g and relaxations by alkaloids are expressed as a percentage of inhibition of the tension obtained with the contractile agent. The concentration needed to produce 50% of the maximum relaxation (IC_{50}) was calculated for each preparation from a least squares regression analysis of all points between 20–80% of the maximal response.

In a separate set of experiments, agonist concentration-response curves were generated by adding increasing concentrations of the agonist cumulatively to the bath (0.5 log unit increments) and allowing each concentration to produce a peak response before addition of the next concentration. Agonist potency was expressed as the pD_2 value. When alkaloids were used, each concentration of the alkaloid was added to the bath and allowed to equilibrate with the tissue for 60 min before addition of the agonist. In all cases, only one alkaloid concentration was tested on each tissue. Dose-dependent contraction curves for the agonists in the presence of alkaloid were related to the control dose-response curve, of which the maximum response was taken as 100%. pA_2 values were obtained according to Arunlakshana & Schild (1959). Antagonism was taken to be competitive when the Schild regression slope did not differ significantly ($P>0.05$) from unity.

Finally another series of experiments was performed in KH buffer in order to determine the inhibitory action of alkaloids on the contraction induced by caffeine at 37°C. An initial NA (10 μM)-induced contraction (NA_1) was obtained and, following a 60 min incubation period in KH solution, a contractile response to caffeine (60 mM) was induced: CAF_1. After washing, a second 60-min incubation period in the presence of different doses of alkaloid allowed us to determine the inhibitory effect of alkaloids on a second caffeine-induced contraction (CAF_2). Both of the caffeine-induced contractile responses, CAF_1 and CAF_2 are expressed as a percentage of the initial contraction induced by noradrenaline (NA_1).

Guinea-pig trachea

Guinea-pig tracheae were removed after the animals had been killed, as described previously, and were placed in KH buffer. Following removal of adhering fat and connective tissue, the tracheae were cut into rings of approximately 4–5 mm. In all experiments, the epithelium was removed by gently rubbing the luminal surface (over both the smooth muscle and cartilage areas) with a cotton-tipped applicator (Devillier *et al.*, 1988). The rings were then suspended in 10 ml organ baths containing KH at 37°C, gassed with 95% O_2 and 5% CO_2 and equilibrated under an initial tension of 2 g. After equilibration for 1 h, the resting tension was 1.91 ± 0.26 g ($n=20$). Tension was measured isometrically as described for aortic rings.

In all experiments, preparations were first tested for maximal tension with acetylcholine (ACh, 1 mM), then allowed to rest for at least 1 h during which washing was performed every 15 min.

The tracheal strips were then contracted to 70–90% of maximal contraction by addition of acetylcholine (100 μM) or histamine (10 μM). When a stable contraction had been obtained, a cumulative concentration-response curve to *S*-(+)-boldine (0.01 μM–100 μM) or papaverine (0.01 μM–100 μM) was obtained by adding increasing concentrations of the alkaloid at 15–20 min intervals. Once the concentration-response curve was completed, theophylline (3 mM) was added to the bath to determine maximal relaxation. Similar to the experiments carried out in guinea-pig aorta, relaxation responses are expressed as a percentage of inhibition of the tension induced by ACh or histamine. The IC_{50} values were calculated from a least squares regression analysis of all points between 20–80% of the maximal response.

Inositol phosphates determination

The method used for determination of total inositol phosphate accumulation was adapted from that of Berridge *et al.* (1982) and Irvine *et al.* (1985). Briefly, the guinea-pig thoracic aortae (8 animals were killed) were exposed to KH containing 2 μCi of myo-[2-^{3}H]-inositol ml^{-1} buffer for 4 h at 37°C and gassed with a mixture of 95% O_2 plus 5% CO_2. After this incubation, the tissue was washed twice with 45 ml KH buffer. Each aorta was cut into four rings which were placed in 4 individual tubes containing 1 ml KH buffer. This procedure was repeated with all guinea-pig aortae to obtain, finally, 4 individual tubes with 8 different samples (0.1–0.2 g) which were incubated at 37°C for 30 min. LiCl (10 mM) was added 30 s before noradrenaline (10 μM) or buffer solution (control) in order to inhibit metabolism of inositol monophosphates (Berridge *et al.*, 1982). Tissues were then incubated with saline or alkaloid (*S*-(+)-boldine: 30 μM or papaverine: 10 μM) for 15 min before stimulation. The samples were stimulated for 15 min with noradrenaline (10 μM) in the absence or presence of the alkaloid. Stimulation was stopped by placing the tissue in a cold water bath (4°C) and by addition of 1.5 ml of a cold mixture of chloroform/methanol/HCl 10 N (100 : 200 : 4, v/v/v) with vigorous shaking. The samples were centrifuged (4000 *g*) for 15 min at 4°C. The aqueous phases were brought to pH 4 with 50 μl of ammonium formate 1.2 M and stored at −20°C until analysis.

The separation of inositol phosphates (IPs) was performed by modifying the method of Irvine *et al.* (1985), by a high performance liquid chromatography (h.p.l.c.) ion-exchange system, using a 0.46 cm × 25 cm Partisil SAX 10 high pressure anion exchange column (Shandon, Cergy-Pontoise, France), flow rate 1.3 ml min^{-1}. The aqueous samples (0.5 ml) were loaded onto the column. Distilled water was then allowed to flow for 6 min to elute inositol and over 24 min a linear gradient was passed through the column rising from 0% to 100% of buffer B (potassium phosphate 1.0 M buffered for pH 3.7 with orthophosphoric acid). Radioactivity was detected by a Flow-One on-line radioactivity flow detector (Packard, Meriden, U.S.A.) equipped with a 2-ml liquid flow cell. Retention times were 15 and 18 min for IP_1 and IP_2, respectively. In this tissue, IP_3 was not detected after a 15 min-stimulation period.

The areas of the reconstructed peaks expressed in d.p.m. (sensitivity 2×10^6 d.p.m. μCi^{-1}) were related to the total [^{3}H]-inositol incorporated into the tissue. Inhibitions induced by alkaloids are expressed as a percentage of the maximal [^{3}H]-inositol phosphates accumulation induced by NA (10 μM) in the absence of alkaloid.

Statistical analysis

All values in the text are expressed as the means ± s.e.mean of five or more preparations (n) obtained from different animals. Test of significance was performed by use of Student's t test for unpaired or paired data, as appropriate, and by ANOVA; P values less than 0.05 were considered to be significant.

Drugs and solutions

The drugs used were: *S*-(+)-boldine hydrochloride, papaverine hydrochloride, (−)-noradrenaline bitartrate, acetylcholine chloride, histamine hydrochloride, prazosin hydrochloride and sodium nitroprusside dihydrate (Sigma Chemical Co); phentolamine methanesulphonate (Ciba-Geigy); anhydrous caffeine (Sandoz); theophylline (Bruneau et Cie., Paris). All agents were dissolved in deionized water, except caffeine which was dissolved in KH buffer. All solutions were prepared daily and the pH was tested. All chemicals used were of analytical grade.

Myo-[2-^{3}H]-inositol with PT6-271 (specific radioactivity, 10–20 Ci mmol^{-1}) was purchased from Amersham International (Amersham, Buckinghamshire, U.K.).

Results

α_1-Adrenoceptor-mediated antagonism of alkaloids and prazosin in guinea-pig aorta

Guinea-pig isolated aortae were incubated in Ca^{2+}-containing KH at 37°C and 90 min later, addition of noradrenaline (NA, 10 μM) induced a sustained contraction (1.64±0.16 g, $n=14$). This contractile response was inhibited dose-dependently by addition of cumulative concentrations of *S*-(+)-boldine (0.03–100 μM) or papaverine (0.03–100 μM) (Figure 2a); the IC_{50} values are summarized in Table 1.

In another set of experiments, dose-response curves for noradrenaline (0.01–100 μM) were obtained in the absence and presence of different concentrations of alkaloids. In control experiments the pD_2 value calculated for noradrenaline was 5.74±0.05 ($n=14$). Pretreatment with different doses of *S*-(+)-boldine (1–30 μM) for 60 min produced parallel rightward shifts of concentration-response curves to NA without affecting the maximum responses (Figure 3a). The Schild plot for the antagonistic effect of *S*-(+)-boldine clearly shows that the slope of the regression line was not significantly different from unity (slope = 1.10±0.18, $n=5$) and the pA_2 value calculated was 5.64±0.08 ($n=5$). Similar results were observed with prazosin (1–100 nM) (Figure 3b) which acted as a competitive antagonist (Schild regression slope = 0.94±0.10, $n=8$) with a pA_2 value of 8.56±0.24 ($n=8$).

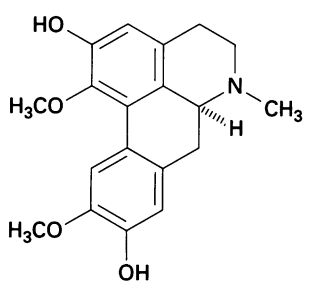

Figure 1 Chemical structure of *S*-(+)-boldine.

Conversely, papaverine (1–30 μM) antagonized non-competitively the NA-induced contractions with depression of the maximal response (Figure 3c).

Antagonism by the alkaloids of voltage-dependent calcium channels

Another series of experiments were performed in order to determine the inhibitory effect of the alkaloids on the extracellular Ca^{2+}- entry-induced responses. Aortic smooth muscle was precontracted by a KCl depolarizing solution (60 mM) to a tension of 1.62±0.12 g ($n=19$). In preliminary experiments, the KCl-induced contraction was elicited in the presence of phentolamine (10 μM) in order to determine a possible participation of noradrenaline released by depolarization, but no significant differences were found between that in the absence of phentolamine (data not shown), so phentolamine was not added to the bath in the following experiments.

In these experimental conditions, only papaverine (0.1–100 μM), not *S*-(+)-boldine (0.1–300 μM), was able to inhibit completely the contractile response induced by 60 mM KCl (see Figure 2b). The E_{max} and IC_{50} values and the IC_{50} (KCl)/IC_{50}(NA) ratio for each drug tested are summarized in Table 1.

In Ca^{2+}-free high-K^+ (60 mM) medium, addition of Ca^{2+} (1 mM) to the bath induced a gradual increase in tension. The maximal tension reached was 1.44±0.17 g ($n=15$) and the concentration-response curves for the relaxation elicited after addition of cumulative doses of *S*-(+)-boldine (0.1–300 μM) or papaverine (0.1–100 μM) were similar to those obtained with KCl 60 mM (Figure 2c; Table 1).

Finally, the effects of both alkaloids were evaluated against a concentration-response curve to Ca^{2+} (0.03–3 mM) in a Ca^{2+}-free high-K^+ (60 mM) medium. Results obtained are presented in Figure 4. In this case, *S*-(+)-boldine (30–300 μM) and papaverine (1–30 μM) both inhibited the contractile responses to Ca^{2+} with depression of the maximal response.

Modification of caffeine-induced contraction by preincubation with different concentrations of the alkaloids

In order to clarify the possible action of *S*-(+)-boldine and papaverine on Ca^{2+} release from internal storage sites, an experimental procedure was designed with caffeine as agonist. In Ca^{2+}-containing KH at 37°C, caffeine 60 mM (CAF_1) induced a rapid transient contraction (phasic component) followed by a decrease of the resting tone and a slow sustained contraction (tonic component). The magnitude of each component of the contraction was 22.98±1.04% ($n=52$) for the phasic component and 19.52±1.51% ($n=52$) for the tonic one, both relative to the contraction induced by 10 μM NA (NA_1: see Methods). After an incubation period (60 min) with different doses of the alkaloids, contraction by caffeine was repeated (CAF_2). In these experimental conditions, *S*-(+)-boldine (1–300 μM) did not modify either the phasic or the tonic components of the contraction induced by caffeine (Figure 5a), whereas papaverine (1–100 μM) was able to inhibit partially both contractions at a dose of 100 μM (Figure 5b, Table 2).

Table 1 Inhibitory potencies of alkaloids on sustained contractions induced by different contracile agents in guinea-pig aorta

Alkaloid		*NA* (10 μM)	*KCl* (60 mM)	*Ca*$^{2+}$ (1 mM)	*r*
Boldine	IC_{50} (μM)	1.4±0.2 ($n=7$)	167.3±21.0* ($n=10$)	143.0±15.9* ($n=6$)	119.5
	E_{max} (%)	97.4±3.1	69.0±2.6*	64.7±4.4*	
Papaverine	IC_{50} (μM)	1.6±0.2 ($n=7$)	5.3±0.5* ($n=10$)	5.4±0.3* ($n=7$)	3.3
	E_{max} (%)	107.9±2.7	101.4±2.7	110.6±4.1	

E_{max} = maximal relaxation; r = IC_{50}(KCl)/IC_{50}(NA). Values are means±s.e.mean. *$P<0.001$, significantly different from the corresponding values for NA-induced contraction; n = number of experiments.

Relaxant effects of S-(+)-*boldine on guinea-pig trachea*

The concentration-response curve obtained after addition of cumulative doses of *S*-(+)-boldine (0.01–100 μM) on the sustained contraction induced by ACh (100 μM) in the guinea-pig isolated trachea shows that maximal relaxation induced by *S*-(+)-boldine 100 μM was 13.0±2.0% (n=6). Also, when similar concentrations of *S*-(+)-boldine were added to tracheas contracted by histamine (10 μM), no significant relaxation was observed.

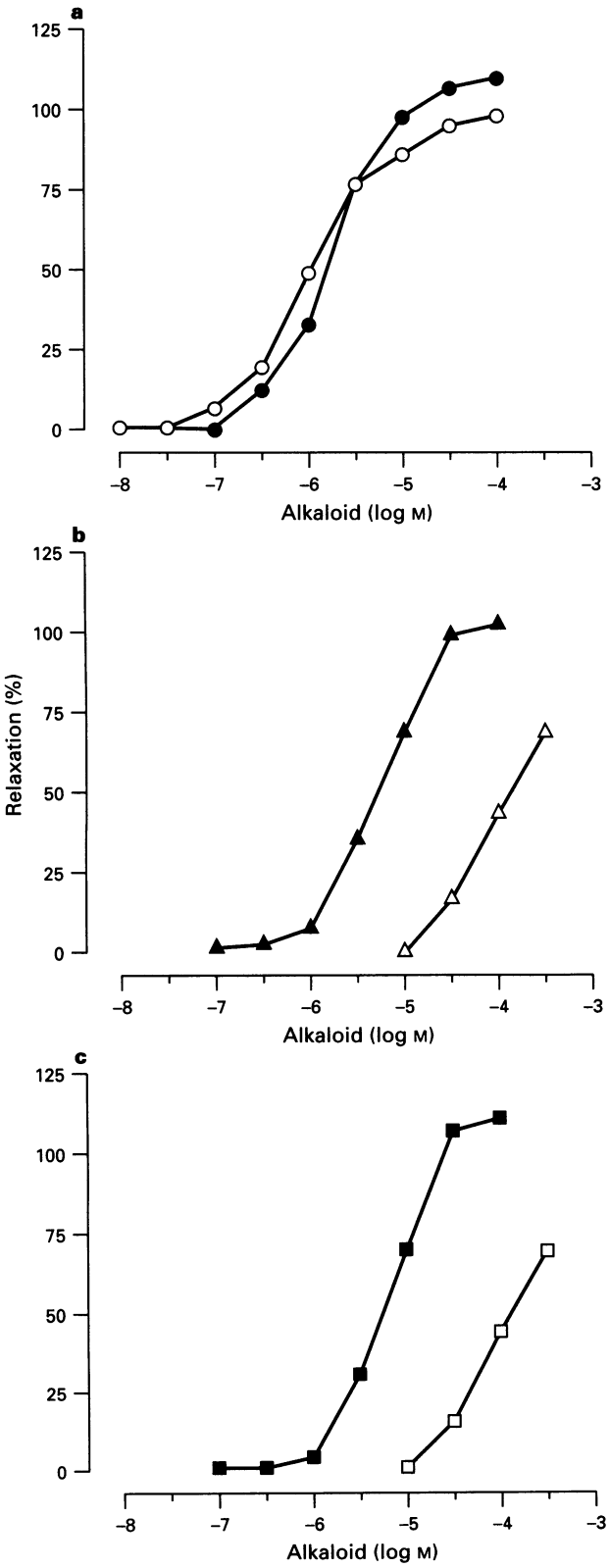

Figure 2 Relaxation dose-response curves obtained after addition of *S*-(+)-boldine (open symbols) or papaverine (closed symbols) in guinea-pig aorta previously contracted by: (a) 10 μM noradrenaline (○, ●); (b) 60 mM KCl (△, ▲); and (c) 1 mM Ca^{2+} (□, ■). Each point is the mean derived from 5–7 experiments with s.e.mean shown by vertical lines.

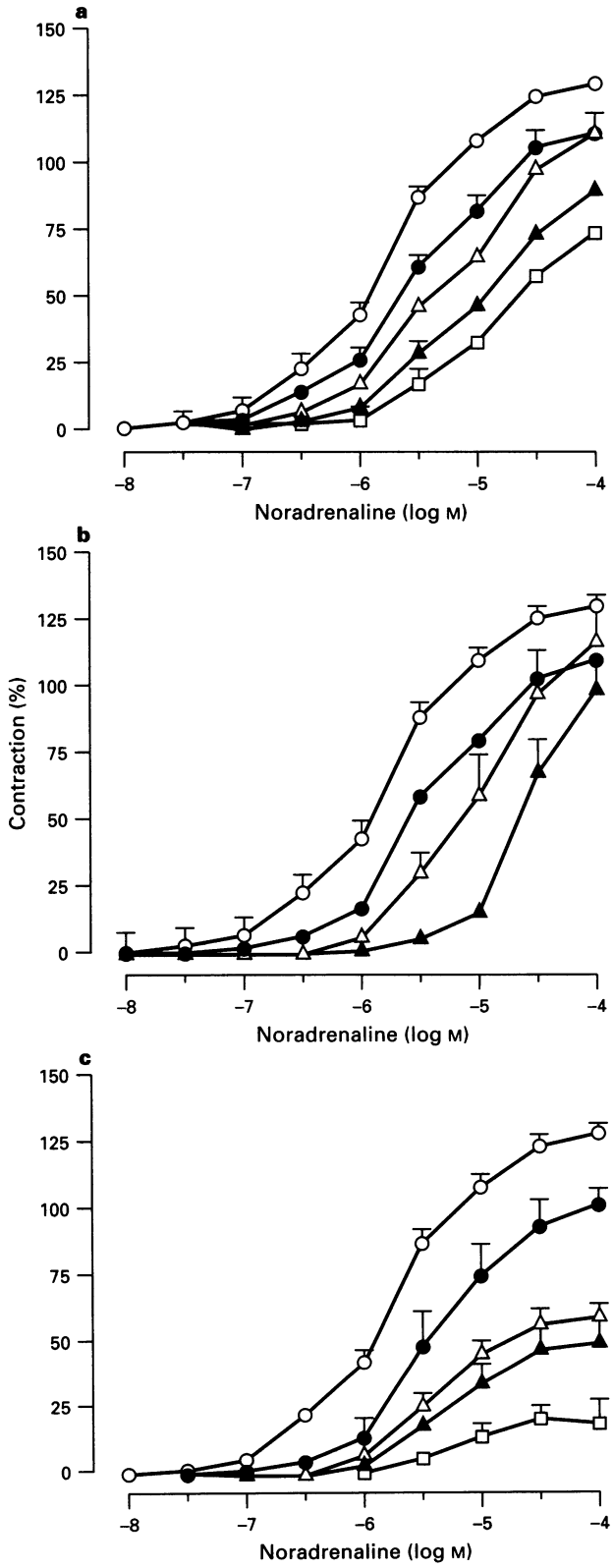

Figure 3 Antagonism of the concentration-response curves to noradrenaline by 60 min pretreatment of denuded aorta with: (a) *S*-(+)-boldine (●, 1 μM; △, 3 μM; ▲, 10 μM; □, 30 μM); (b) prazosin (●, 1 nM; △, 10 nM; ▲, 100 nM); and (c) papaverine (●, 1 μM; △, 3 μM; ▲, 10 μM; □, 30 μM). Each point represents the mean of data from 5–14 experiments and vertical lines show s.e.mean.

In contrast to S-(+)-boldine, papaverine exerted a dose-dependent relaxant effect in the guinea-pig trachea. The IC_{50} values calculated for papaverine on ACh- or histamine-induced contractions were 2.73 ± 0.57 μM ($n=6$) and 2.94 ± 0.32 μM ($n=4$), respectively, and no significant differences were found between these and those previously obtained (Candenas *et al.*, 1990).

Influence of S-(+)-*boldine and papaverine on phosphoinositide metabolism*

Figure 6 shows the effect in guinea-pig aorta of S-(+)-boldine (30 μM) and papaverine (10 μM) on phosphoinositide accumulation induced by NA (10 μM) in normal KH. The doses of S-(+)-boldine (30 μM) and papaverine (10 μM) used in these experiments were those that induced a relaxation of ~90% of the noradrenaline-induced contraction in guinea-pig aorta.

It was found that noradrenaline induced a significant increase in total [^{3}H]-inositol phosphates accumulation of $166.5 \pm 38.6\%$ ($n=5$) (relative to the basal value) which was only markedly inhibited after treatment with S-(+)-boldine. The PI accumulation induced by NA in the presence of S-(+)-boldine was $23.1 \pm 5.0\%$ ($n=5$) of the PI accumulation induced by NA alone. In contrast, papaverine did not affect significantly the response induced by 10 μM NA, only reducing it to $85.4 \pm 6.4\%$ ($n=5$) of the control PI accumulation.

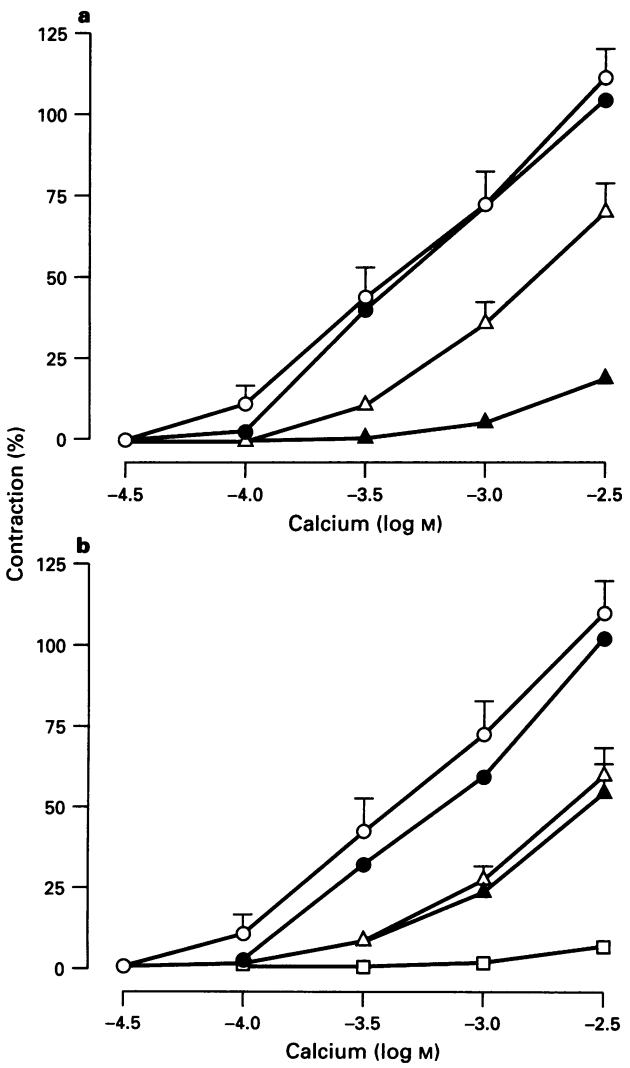

Figure 4 Antagonism of the concentration-response curves to Ca^{2+} by 60 min pretreatment of denuded aorta with: (a) S-(+)-boldine (●, 30 μM; △, 100 μM; ▲, 300 μM); and (b) papaverine (●, 1 μM; △, 3 μM; ▲, 10 μM; □, 30 μM); (○) control responses. Each point represents the mean of data from 4–7 experiments and vertical lines show s.e.mean.

Discussion

In previous studies, we have determined the α_1-adrenoceptor antagonist activity of different compounds with a benzyltetrahydroisoquinoline (BTHIQ) structure closely related to papaverine. All the compounds tested have shown an inhibitory action on α_1-adrenoceptors and Ca^{2+} influx from the extracellular medium without producing changes in the intracellular distribution of this ion (Anselmi *et al.*, 1992; Candenas *et al.*, 1990; Chuliá *et al.*, 1994; 1995a,b; D'Ocón *et al.*, 1989; Ivorra *et al.*, 1992; 1993a). In contrast, papaverine, a benzylisoquinoline alkaloid containing an unsaturated heterocyclic ring, exhibited a relaxant effect mediated by non-specific inhibition of cyclic nucleotide phosphodiesterases (PDE) (Lugnier *et al.*, 1972; Ivorra *et al.*, 1992) and the subsequent increase in adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and modification of intracellular Ca^{2+} distribution. This prompted us to examine the α_1-adrenoceptor antagonist activity of S-(+)-boldine, a BTHIQ alkaloid, in the guinea-pig aorta and to compare its effect with that of papaverine.

In guinea-pig aorta, contraction induced by noradrenaline depends mainly upon release of intracellular Ca^{2+} through PI pathway activation without participation of extracellular Ca^{2+}-entry through voltage-dependent calcium channels (Jenkin *et al.*, 1991). The α-adrenceptor responsible for this contraction has been identified as α_{1A}- (Oriowo, 1994) or α_{1B}-subtype (Veenstra *et al.*, 1992). Nevertheless, mechanisms other than α_1-adrenoceptor activation can be involved in the vascular smooth muscle contraction. Thus, it has been shown that a high K^+ concentration causes a contraction by depolarizing cell membranes and by increasing the influx of Ca^{2+} through L-type voltage-dependent channels that are sensitive to Ca^{2+} antagonists like nifedipine and diltiazem (Godfraind *et al.*, 1986) and, in some vascular tissues, like rat aorta, it has been demonstrated that high K^+ even induces catecholamine release from sympathetic terminal nerves (Ivorra *et al.*, 1993a). In the case of guinea-pig aorta, we have concluded that catecholamine release does not participate to the contraction elicited by depolarization, since the contractile response induced by KCl (60 mM) alone does not differ significantly from that obtained by KCl in the presence of phentolamine 10 μM (data not shown).

The present results show that S-(+)-boldine (0.3–300 μM) and papaverine (0.3–100 μM) are able to inhibit concentration-dependently contractions induced by NA, KCl or Ca^{2+} in guinea-pig aorta with different potencies and selectivities.

Table 2 Effect of alkaloids on caffeine (CAF_2)-induced contraction of guinea-pig aorta

Agonist		*Boldine*	*Papaverine*
CAF_2 without alkaloid	Ph	156.6 ± 11.1	
	T	117.3 ± 13.0	
CAF_2 + alkaloid 1 μM	Ph	129.7 ± 6.3	121.0 ± 17.5
	T	93.7 ± 6.1	118.1 ± 17.3
CAF_2 + alkaloid 10 μM	Ph	146.1 ± 21.1	66.6 ± 8.2**
	T	93.9 ± 10.5	97.6 ± 17.8
CAF_2 alkaloid 100 μM	Ph	125.7 ± 12.1	28.6 ± 7.0**
	T	73.7 ± 18.2	54.1 ± 13.9*
CAF_2 alkaloid 300 μM	Ph	150.1 ± 12.5	
	T	111.3 ± 22.4	

Ph and T represent the phasic and tonic components of the CAF_2-induced contraction. Values are expressed as a percentage of CAF_1 (see Methods). Values are means ±s.e.mean from 4–10 experiments. *$P<0.01$, **$P<0.001$, significantly different from CAF_2 without alkaloid.

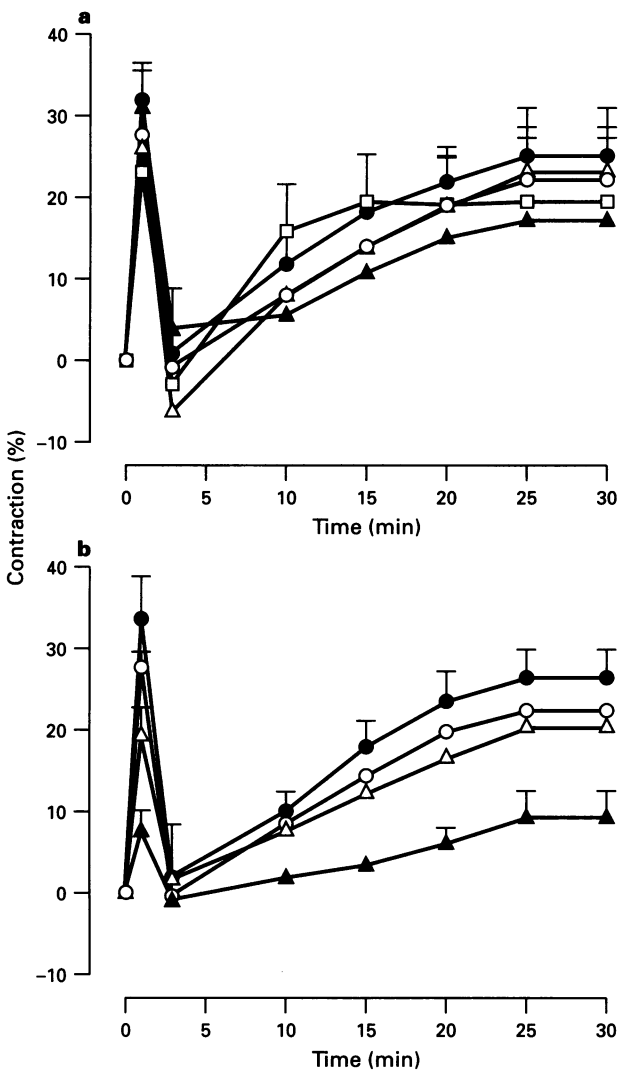

Figure 5 Inhibitory effect of different doses of: (a) *S*-(+)-boldine (●, 1 μM; △, 10 μM; ▲, 100 μM; □, 300 μM); and (b) papaverine (●, 1 μM; △, 10 μM; ▲, 100 μM) on the contraction of guinea-pig isolated aorta induced by caffeine 60 mM. (○) Control response in the absence of the alkaloid. Contractions are expressed as a percentage of the noradrenaline (10 μM)-induced contraction. Each point represents the mean of data from 4–10 experiments and vertical lines show s.e.mean.

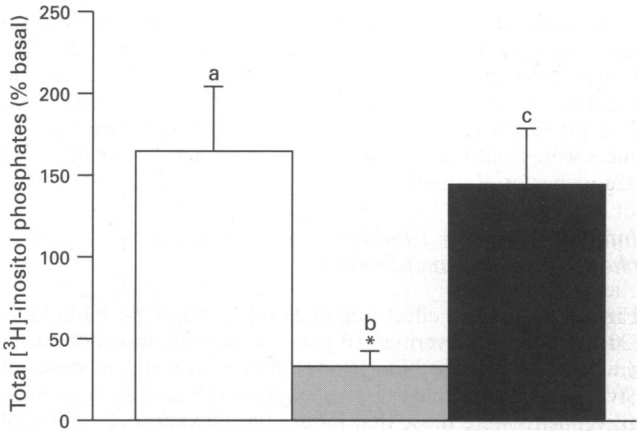

Figure 6 Total [^{3}H]-inositol phosphates production in guinea-pig aorta induced by noradrenaline 10 μM in the absence (a) or presence of *S*-(+)-boldine 30 μM (b) or papaverine 10 μM (c). Results are expressed as mean ± s.e.mean from five experiments. $*P<0.001$, significant inhibition of total [^{3}H]-inositol phosphates accumulation.

Analysis of the IC_{50}(KCl)/IC_{50}(NA) ratios provides information of selectivity (Table 1) and indicates that *S*-(+)-boldine exhibts a greater inhibition of the contractile response induced by NA ($IC_{50} = 1.4 \pm 0.2$ μM) than that induced by KCl or Ca^{2+}. Conversely, papaverine relaxes contractile responses to either NA, KCl or Ca^{2+} in a non-selective manner, as previously described in other tissues: rat uterine (Anselmi *et al.*, 1992), human bronchial (Candenas *et al.*, 1990) and rat vascular (Ivorra *et al.*, 1992) smooth muscle. These findings agree with the results obtained in previous studies where *S*-(+)-boldine interacted with [^{3}H]-prazosin and [^{3}H]-diltiazem binding to rat cerebral cortex, showing a higher affinity for the α_1-adrenoceptor binding site than for the benzothiazepine receptor site (Ivorra *et al.*, 1993a). This selectivity of *S*-(+)-boldine for the α_1-adrenoceptors of the guinea-pig aorta is also supported by the results obtained in guinea-pig trachea where *S*-(+)-boldine is not able to relax the acetylcholine- or histamine-induced contractions, which are predominantly mediated by Ca^{2+} derived from intracellular stores (Advenier *et al.*, 1984) after muscarinic or histamine receptor activation.

Moreover, if we compare the dose-dependent relaxations induced by *S*-(+)-boldine on the KCl-induced contractions in guinea-pig aorta ($IC_{50} = 167.3 \pm 21.0$ μM) (present results) with those in rat aorta ($IC_{50} = 63.2 \pm 9.1$ μM) (Ivorra *et al.*, 1993a), the greater potency observed for *S*-(+)-boldine in this latter tissue is noticeable. It has been suggested that high K^+-induced contractions in guinea-pig aorta are relatively less sensitive to nifedipine than in rat aorta (Jenkin *et al.*, 1991) and this could explain the differences observed with *S*-(+)-boldine on KCl 60 mM-induced contractions in both tissues. In this way, *S*-(+)-boldine could act, at high concentrations, as a calcium antagonist of potential-operated Ca^{2+} channels.

It is of interest to note that the relaxant effects of both alkaloids were present in the guinea-pig aorta in the absence of endothelium. Therefore, the vasorelaxations induced by *S*-(+)-boldine and papaverine are independent of an intact endothelium and not mediated by either endothelium-derived relaxing factor (EDRF) of prostacyclin (PGI_2) (Jaffe, 1985; Vanhoutte *et al.*, 1986).

In order to assess if *S*-(+)-boldine is acting by an intracellular mechanism, not related to α_1-adrenoceptor activation, we designed a new set of experiments with caffeine (60 mM) as contractile agent. The mechanism whereby caffeine causes a contractile response in vascular smooth muscle implies Ca^{2+}-induced Ca^{2+} release from sarcoplasmic reticulum and Ca^{2+} entry from extracellular medium (Sato *et al.*, 1988). The caffeine-induced contraction (CAF_2) was not modified by *S*-(+)-boldine (1–300 μM) whereas papaverine, at the maximal concentration tested (100 μM), inhibited this caffeine response. These results ascertain that the selective inhibitory effect of *S*-(+)-boldine on NA-induced contraction is not attributable to direct inhibition of smooth muscle contractile elements or Ca^{2+} release from internal stores but could be due to antagonism of α_1-adrenoceptors or inhibition of receptor-mediated signal transduction. Hence, to identify the mechanism whereby these two alkaloids act, we, firstly, studied the type of antagonism of α_1-adrenoceptors shown by *S*-(+)-boldine and papaverine and, secondly, the interaction of both compounds with the intracellular levels of inositol phosphates. It is well known that contraction of the vascular smooth muscle of the guinea-pig aorta caused by noradrenaline is associated with receptor-coupled phospholipase C-induced breakdown of phosphatidylinositol with generation of two second messengers: inositol 1,4,5-triphosphate (IP_3) and s,n-1,2-diacylglycerol (Jenkin *et al.*, 1991).

Analysis of the cumulative concentration-response curves obtained for noradrenaline in the absence and presence of alkaloids (Figure 3) indicates that the two alkaloids interact

differently. The dose-response curve for NA was shifted to the right in both cases but calculation of Schild regression lines only gave a slope not significantly different from unity in the case of *S*-(+)-boldine. Thus, the antagonism btween *S*-(+)-boldine and α_1-adrenoceptors was competitive with a pA_2 value of 5.64 whereas papaverine showed non-competitive antagonism. In our functional study, we also determined the type of antagonism induced by prazosin, a potent α_1-adrenoceptor antagonist. This agent antagonized NA-induced contractions in a competitive manner with a pA_2 of 8.56 which is similar to the pA_2 value previously obtained for prazosin against NA in this same tissue (Oriowo & Ruffolo, 1992). It is of a great interest to note that in rat aorta denuded of endothelium *S*-(+)-boldine showed non-competitive antagonism against NA (Ivorra *et al.*, 1993a) while prazosin acted again as a competitive antagonist of the contraction induced by NA (Aboud *et al.*, 1993; Ko *et al.*, 1994). In rat aorta, the α_1-adrenoceptor has been variously classified as α_{1B} (Han & Minneman, 1990), both α_{1A} and α_{1B} (Piascik *et al.*, 1991) or non α_{1A}, non α_{1B}-subtype (the putative α_{1D}-adrenoceptor) (Aboud *et al.*, 1993). The α_1-adrenoceptor present in the guinea-pig aorta has been defined as either an α_{1A}- (Oriowo, 1994; Oriowo & Ruffolo, 1992) or α_{1B}-subtype (Oriowo & Ruffolo, 1992; Veenstra *et al.*, 1992), both of them couple to the release of intracellular Ca^{2+} through an increased production of inositol phosphates (Jenkin *et al.*, 1991). It is probable that *S*-(+)-boldine shows non-competitive antagonism in rat aorta due to an interaction with a heterogeneous receptor population subserving the same contractile response. Whereas in vascular smooth muscle of guinea-pig aorta this alkaloid acts as a competitive α_1-adrenoceptor antagonist because in this tissue only a hŏmogeneous receptor population exists.

Analysis of the action of *S*-(+)-boldine on the formation of [^{3}H]-inositol phosphates induced by noradrenaline 10 μM in the guinea-pig aorta shows that this benzylisoquinoline alkaloid, at a concentration of 30 μM which relaxes almost completely the NA-induced contraction of the guinea-pig aorta, is able to block the PI formation pathway linked to α_1-adrenoceptor activation. These data support the hypothesis that *S*-(+)-boldine is an α_1-adrenoceptor antagonist in guinea-pig aorta acting on a homogeneous receptor population and, also, corroborate the fact that α_1-adrenoceptor activation causes phosphoinositide hydrolysis in this tissue.

The different behaviour of *S*-(+)-boldine and papaverine at the α_1-adrenoceptor of the guinea-pig aorta can be explained on the basis of their structural features. The BTHIQ alkaloids such as *S*-(+)-boldine, have an sp^3-like hybridize nitrogen atom, a partially flexible tetrahydroisoquinoline ring and no chiral centre. In contrast, papaverine, a benzylisoquinoline alkaloid, has an sp^2-like nitrogen atom, a planar isoquinoline ring and no chiral centre. These structural features determine the geometry of both molecules and could explain their different mechanisms of action on the α_1-adrenoceptor subtype of the guinea-pig aorta. This hypothesis is supported by similar results previously observed when comparing another aporphine alkaloid closely related to *S*-(+)-boldine, *S*-(+)-glaucine, with papaverine in the rat aorta (Ivorra *et al.*, 1992).

The results obtained in this study suggest that aporphine compounds could be considered as putative antihypertensive drugs due to their adrenoceptor antagonist properties. Nevertheless, future studies carried out *in vivo* models are required to determine the action of this structural class of compounds on the cardiovascular system and its effects on arterial pressure and heart rate.

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