J. Pharm. Pharmacol. 1998, 50: 1267–1273
Received April 2, 1998
Accepted June 24, 1998

α-Adrenoceptor Interaction of Tetrandrine and Isotetrandrine in the Rat: Functional and Binding Assays

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

Departamento de Farmacología, Facultad de Farmacia, Universitat de València, Avda. Vicent Andrés Estellés s/n 46100, Burjassot, València, Spain

Abstract

The action of 1*S*,1′*S*-tetrandrine, a bisbenzyltetrahydroisoquinoline alkaloid, on α_1-adrenoceptors has been compared with that of its isomer 1*R*,1′*S*-isotetrandrine. The work includes binding assays to analyse the affinity of these products for the [^{3}H]prazosin binding site of rat cerebral cortical membranes and functional studies on rat isolated aorta to examine the effects of both alkaloids on intracellular calcium processes related or not to α-adrenoceptor activation.

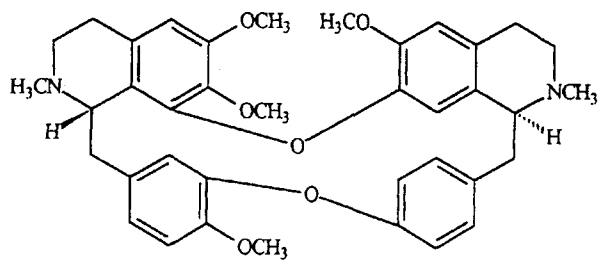
A radioligand receptor-binding study showed that both compounds interacted with the α_1-adrenoceptors displacing [^{3}H]prazosin from the specific binding site. The K_i values (inhibition constants) were $0{\cdot}69 \pm 0{\cdot}12$ and $1{\cdot}6 \pm 0{\cdot}4$ μM for tetrandrine and isotetrandrine, respectively. The functional studies showed that both alkaloids concentration-dependently inhibited noradrenaline-induced contraction in Ca^{2+}-free solution (IC50 values, i.e. the concentrations needed to induce 50% inhibition, were 252·8 and 174·9 μM for tetrandrine and isotetrandrine, respectively), the spontaneous contractile response elicited by extracellular calcium after depletion of noradrenaline-sensitive intracellular stores (increase in resting tone; IC50 values 11·6 and 19·6 μM for tetrandrine and isotetrandrine, respectively) and the refilling of intracellular Ca^{2+} stores sensitive to noradrenaline (IC50 values 7·4 and 14·9 μM for tetrandrine and isotetrandrine, respectively).

The results show that tetrandrine and isotetrandrine interact with α_1-adrenoceptors by displacing the [^{3}H]prazosin binding site and that both compounds inhibit mainly the Ca^{2+}-dependent process and have less action on α_1-adrenoceptors. Tetrandrine is more potent than isotetrandrine.

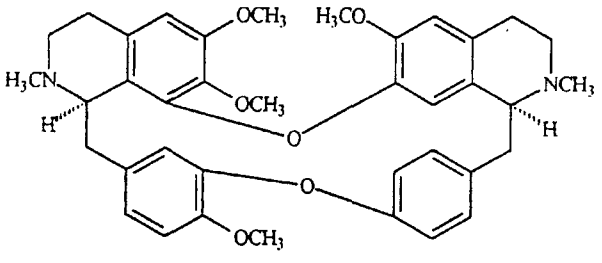
Tetrandrine, a bisbenzyltetrahydroisoquinoline alkaloid isolated from the Chinese medicinal plant *Stephania tetrandra*, is the most potent member of a new class of calcium-entry blockers of natural origin. It has been found to inhibit T- and L-type Ca^{2+} channels in a number of cell types, and binding studies have shown that it interacts at the benzothiazepine recognition site in the Ca^{2+} channel (King et al 1988; Felix et al 1992; Wang & Lemos 1995). Chemically, tetrandrine is a dimer with two benzylisoquinoline subunits condensed head-to-head, tail-to-tail fashion with 1*S*,1′*S* stereochemistry at the chiral isoquinoline carbon (Schiff 1987) (Figure 1).

Correspondence: E. Anselmi, Departamento de Farmacología, Facultad de Farmacia, Universitat de València. Avda. Vicent Andrés Estelles s/n 46100. Burjassot, València, Spain.

In a previous study, Anselmi et al (1994) examined the action of tetrandrine on rat aorta and compared it with the relaxant action of its isomer 1*R*,1′*S*-isotetrandrine, which differs from tetrandrine only in the stereochemistry at the chiral centres. In that study it was observed that tetrandrine and isotetrandrine relax the contractile response elicited by depolarizing solution (KCl 80 mM) or noradrenaline (1 μM), and in Ca^{2+}-free solution at the highest concentration tested (300 μM) they inhibited the contraction induced by noradrenaline. They did not, however, affect the contraction induced by caffeine. It has been suggested that in rat aorta the noradrenaline-induced release of intracellular Ca^{2+} is attributable to α_1-adrenoceptor activation (Noguera & D'Ocón 1992) whereas the mechanism of caffeine-induced Ca^{2+}-release is different. Both alkaloids could therefore



Tetrandrine



Isotetrandrine

Figure 1. The chemical structures of tetrandrine (1*S*,1′*S* isomer) and isotetrandrine (1*R*,1′*S* isomer).

act at the α_1-adrenoceptor level. To confirm this hypothesis the current work includes binding studies for determination of the interaction of the alkaloids with the α_1-adrenoceptor by examining their effects on [^{3}H]prazosin binding to rat cerebral cortical membranes. The work also includes functional studies on rat isolated aorta to examine in greater detail the effects of different concentrations of both alkaloids on noradrenaline-induced contraction in Ca^{2+}-free solution and on the process of refilling of intracellular calcium stores previously depleted with noradrenaline (α_1-adrenoceptor activation) or caffeine.

Some of these data have already been presented in abstract form (Anselmi et al 1996).

Materials and Methods

Drugs and solutions

(−)-Noradrenaline L-tartrate was from Merck and anhydrous caffeine from Sigma (St Louis, MO). Tetrandrine was a gift from Dr D. Fang (Department of Pharmacology, Tongji Medical University, Wuhan, China) and isotetrandrine was isolated from *Limaciopsis loangensis* by the method of Cavé et al (1979). Prazosin and phentolamine were from Sigma, and [^{3}H]prazosin (20·3 Ci mmol^{-1}) from Amersham International (Bucks, UK). Other reagents were of analytical grade.

Caffeine was dissolved in Ca^{2+}-free Krebs solution, other drugs in distilled water. All solutions were prepared daily and the pH was tested.

The composition of the Krebs solution was (mM): NaCl 118, KCl 4·75, $CaCl_2$ 1·8, $MgCl_2$ 1·2, KH_2PO_4 1·2, $NaHCO_3$ 25, glucose 11. The composition of the Ca^{2+}-free solution was identical except that $CaCl_2$ was omitted and EDTA (0·1 mM) was added.

Binding study

Preparation of membranes. Female Wistar rats, 180–200 g, were decapitated and the brains rapidly removed. The cerebral cortex was homogenized in 10 vols (w/v) ice-cold buffer (Tris HCl 5 mM, sucrose 250 mM and EDTA 1 mM; pH 7·5 at 25°C) using an Ultra-Turrax (2 × 15 s). The homogenate was centrifuged for 10 min at 1000 *g*, the pellet was discarded and the supernatant was centrifuged at 50 000 *g* for 15 min at 4°C. The final pellet was resuspended in assay buffer and stored at −70°C for later use. All membrane-preparation procedures were conducted at 4°C.

[^{3}H]prazosin-binding studies. Binding of [^{3}H] prazosin was measured in samples of diluted membranes incubated in 50 mM Tris buffer (pH 7·5) with [^{3}H]prazosin (0·2 nM) and in the absence or presence of 17–20 concentrations of the indicated agents. The incubation volume was 1 mL (approx. 250 μg protein/tube). The assay tubes were incubated for 45 min at 25°C and the binding reactions were then terminated by rapid vacuum filtration using a Brandel cell harvester (M24R) with fibre-glass filters (Schleicher and Schuell, No. 30) presoaked in 0·3% polyethylenimine for 5 min. The filters were then washed with ice-cold 50 mM Tris-HCl buffer, pH 7·5 (4 × 4 mL) and the radioactivity bound to the filters was determined by liquid-scintillation counting. Non-specific binding was defined as binding in the presence of 10 μM phentolamine.

Proteins were assayed according to the method of Bradford with γ-globulin as standard (Bradford 1976). All results were obtained in triplicate. Displacement curves were analysed by the weighted least-squares iterative curve-fitting program, Ligand (Munson & Rodbard 1980), and inhibition constants (K_i) were calculated by use of the formula of Cheng & Prussoff (1973).

Functional study

Rings of thoracic aorta (denuded of endothelium) from male Wistar rats, 200–220 g, were prepared and mounted as described by Furchgott & Zawadzki (1980). 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).

Endothelium-denuded aortic rings were prepared by rubbing the entire intimal surface. The absence of relaxant response after acetylcholine (100 μM) addition to preparations contracted with noradrenaline (1 μM) indicated the absence of functional endothelium from all the rings (Furchgott & Zawadzki 1980).

Analysis of results

Contractions in physiological solution were expressed as mg of developed tension and, when elicited in Ca^{2+}-free medium, as a percentage of noradrenaline- or caffeine-induced contraction obtained in normal physiological solution. The increase in resting tone (IRT) was also expressed as a percentage of the noradrenaline-induced contraction in normal physiological solution. The concentration needed to induce 50% inhibition (IC50) was obtained by non-linear regression analysis (Graph Pad Software; San Diego, CA, USA) and results are presented as the means of n determinations. It was, however, impossible to calculate the standard error of the mean (s.e.m.).

Results

Binding assays

The binding of [^{3}H]prazosin to the rat cerebral cortex membranes was specific, saturable and of high affinity. Non-linear regression analysis of the saturation data was consistent with the presence of a single population of binding sites. The K_D (affinity) and B_{max} (total number of receptors) values derived were $0{\cdot}11 \pm 0{\cdot}02$ nM and $132{\cdot}5 \pm 7{\cdot}2$ fmol (mg protein)$^{-1}$, respectively (Sallés & Badía 1994). The specific binding of [^{3}H]prazosin at a concentration of 0·2 nM represented approximately 90% of the total binding.

The interaction of tetrandrine and isotetrandrine with [^{3}H]prazosin binding is shown in Figure 2. Both alkaloids completely inhibited [^{3}H]prazosin binding to cortical membranes, with inhibition constants (K_i) of $0{\cdot}69 \pm 0{\cdot}12$ and $1{\cdot}66 \pm 0{\cdot}4$ μM for tetrandrine and isotetrandrine, respectively (Table 1).

That the pseudo-Hill coefficient (slope factor) was not significantly different from unity

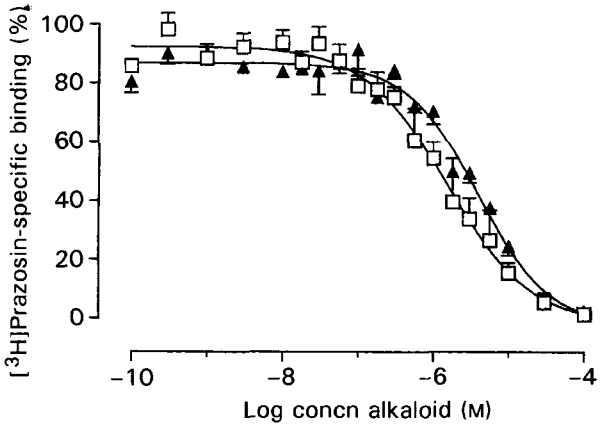


Figure 2. Displacement of the specific binding of [^{3}H]prazosin (concentration–response curve) to the rat cerebral cortex by tetrandrine (□) or isotetrandrine (▲). Each point is the mean of results from 5 or 6 experiments performed in triplicate; the s.e.m. is shown by the vertical lines.

($n_H = 0{\cdot}89 \pm 0{\cdot}08$ and $0{\cdot}97 \pm 0{\cdot}07$ for tetrandrine and isotetrandrine, respectively) suggests direct competition between the alkaloids and the radioligand for a single common binding site.

Functional assays

Effects of alkaloids on the basal tone of the aorta. Contractile response was obtained by addition of 1 μM noradrenaline after washed, stable basal tone was obtained. Subsequent addition of cumulative amounts (0·1–300 μM) of tetrandrine or isotetrandrine did not modify the tone of the aorta rings.

Effect of alkaloids on noradrenaline-induced contraction in Ca^{2+}-free solution. In Krebs solution noradrenaline (1 μM) evoked a maximum contraction. The magnitude of the contractile response of rat aortic tissue was $849{\cdot}57 \pm 160{\cdot}25$ mg (n = 7). After 15 min in Ca^{2+}-free solution (Figure 3), addition of noradrenaline induced a biphasic contraction (NA_1). The magnitude of these contractions was $30{\cdot}2 \pm 3{\cdot}3$% (n = 7). A second application of noradrenaline (1 μM) in Ca^{2+}-free solution ($NA_{1'}$) evoked no response. Upon re-exposure of the tissues to a Ca^{2+}-containing solution for 20 min, an increase in the resting tone was observed. This increase was $30{\cdot}68 \pm 2{\cdot}8$ % (n = 7) relative to noradrenaline-induced contractions in physiological solution. Returning the tissues to a Ca^{2+}-free solution reduced the tension to baseline and further application of noradrenaline (NA_2) 15 min later induced a contraction similar in size to the first contraction elicited in Ca^{2+}-free solution (NA_1).

With the aim of ascertaining the influence of the alkaloids on Ca^{2+}-release from intracellular storage sites sensitive to noradrenaline, concentration–response curves for inhibition were obtained by

Table 1. Inhibitory potency of tetrandrine and isotetrandrine.

Agent	Functional studies					Binding studies
	KCl IC50 (μM)	NA IC50 (μM)	NA_2 (−Ca) IC50 (μM)	IRT_2 IC50 (μM)	NA_3 (−Ca) IC50 (μM)	[^{3}H]Prazosin K_i (μM)
Tetrandrine	13·58 ± 2·1† (n=7)	29·54 ± 0·6† (n=5)	252·8 (n=3–5)	11·6 (n=3–6)	7·4 (n=4–6)	0·69 ± 0·12 (n=5)
Isotetrandrine	23·30 ± 2·0*† (n=5)	22·30 ± 3·0† (n=5)	174·9 (n=3–5)	19·6 (n=3–5)	14·9 (n=4–6)	1·66 ± 0·40 (n=5)

KCl=effect on the contraction induced by 80 mM potassium chloride; NA=effect on the contraction induced by 1 μM noradrenaline in Ca^{2+}-containing solution; NA_2 (−Ca)=effect on the contraction induced by 1 μM noradrenaline in Ca^{2+}-free solution (see Figure 3); IRT_2=effect on the increase in the resting tone after depletion of intracellular Ca^{2+} pools sensitive to noradrenaline (see Figure 4a); NA_3 (−Ca)=effect, in Ca^{2+}-free solution, on the refilling of intracellular Ca^{2+} pools sensitive to noradrenaline (see Figure 4a); K_i=inhibitory potency of the alkaloids on [^{3}H]prazosin-binding to specific receptors of rat cerebral cortex membranes. IC50 is the concentration needed to induce 50% inhibition. All values are means ± s.e.m.; n=number of experiments. *$P < 0.05$ significantly different from result for tetrandrine. †Anselmi et al 1994.

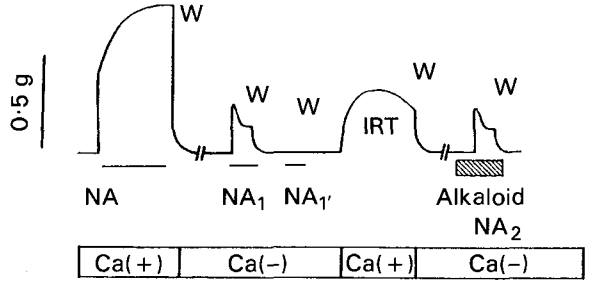


Figure 3. Experimental procedure used to study the effect of alkaloids on noradrenaline-induced contraction in Ca^{2+}-free solution. NA_1=addition of the agonist after 15-min incubation in Ca^{2+}-free solution. $NA_{1'}$=second addition of the agonist after washing (W) in Ca^{2+}-free solution. NA_2=addition of the agonist in the presence or absence of different concentrations (3–300 μM) of the alkaloid, after 20-min resting period in Krebs bicarbonate solution (Ca^{2+} 1·8 mM) and 15 min in Ca^{2+}-free solution. IRT=increase in resting tone.

pre-incubation with tetrandrine or isotetrandrine, 15 min before noradrenaline (1 μM) in Ca^{2+}-free solution (Figure 3) (NA_2). The IC50 values obtained were 252·8 μM (n=6) for tetrandrine and 174·9 μM (n=5) for isotetrandrine (Table 1)

Effect of alkaloids on the increase in resting tone (IRT) of aorta and refilling of intracellular Ca^{2+} stores sensitive to noradrenaline. To investigate the possible modification by tetrandrine or isotetrandrine of the spontaneous contractile response elicited by extracellular calcium after depletion of noradrenaline-sensitive intracellular stores (increase in resting tone, IRT) and of the refilling of intracellular Ca^{2+} pools sensitive to noradrenaline, concentration–response curves for inhibition were obtained by additing the alkaloids 15 min before and during the genesis of the IRT (Figure 4a). Both alkaloids induced concentration-dependent inhibition. The IC50 values obtained are shown in Table 1. Complete inhibition of this contractile process was observed at the highest concentration (300 μM). These alkaloids also inhibited the refilling of the internal Ca^{2+} pools sensitive to noradrenaline. The IC50 values are shown in Table 1. At the maximum concentration assayed (300 μM) both alkaloids completely inhibited the response to noradrenaline in Ca^{2+}-free solution (NA_3).

Influence of alkaloids on the refilling of intracellular Ca^{2+} stores sensitive to caffeine. At 25°C caffeine (10 mM) induced a rapid transient contraction in normal Krebs solution (Figure 4b; Table 2). Addition of caffeine after 15 min exposure to Ca^{2+}-free solution resulted in a phasic contraction

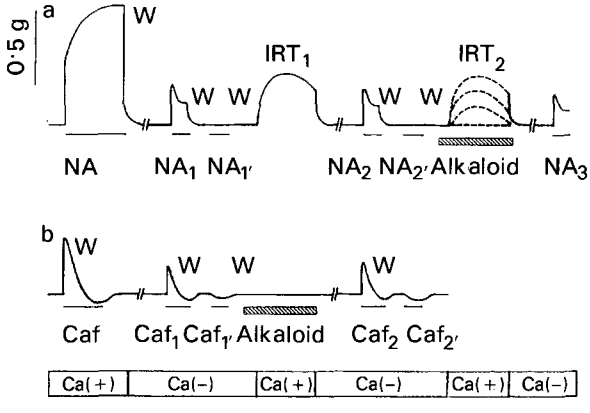


Figure 4. Experimental protocol used to analyse the influence of alkaloids on the increase in resting tone (IRT) of aorta and on the refilling of intracellular Ca^{2+} stores sensitive to noradrenaline (a) or caffeine (b). NA_1 or Caf_1=addition of the agonist after 15-min incubation in Ca^{2+}-free solution. $NA_{1'}$ or $Caf_{1'}$=second addition of the agonist after washing (W) in Ca^{2+}-free solution. NA_2 or Caf_2 (or NA_3)=addition of the agonist after 20-min resting period in Krebs solution (Ca^{2+}-loading period) and 15-min incubation in Ca^{2+}-free solution. In the experiment designed to assess the effects of the alkaloids on the increase in the resting tone and on the refilling of intracellular Ca^{2+} stores sensitive to noradrenaline or caffeine, the aorta was pretreated with different concentrations of these agents (3–300 μM) both 15 min before and during the Ca^{2+}-loading period.

Table 2. Contractile response to caffeine, in Ca^{2+}-free solution, of tissues treated with the agents during the refilling of Ca^{2+} stores.

Agent	Dose (μM)	n	Caf_1 (%)	Caf_2 (%)
Control	–	6	61·2±5·2	61·9±5·2
Tetrandrine	100	3	44·1±9·1	21·7±11·7
	300	4	55·3±3·1	19·6±3·6*
Isotetrandrine	100	3	39·9±13·4	30·5±8·2
	300	6	35·8±7·6	39·9±13·4

Caf_1 =contraction response to caffeine when added after 15-min incubation in Ca^{2+}-free solution. Caf_2=contraction response to caffeine when added after 20-min resting period in Krebs solution (Ca^{2+}-loading period) and then 15-min incubation in Ca^{2+}-free solution. Contractions are expressed as a percentage of the caffeine-induced contraction in physiological solution. All values are means±s.e.m.; n=number of experiments. *$P < 0.01$ significantly different from result for Caf_1. See Figure 4b.

(61·2±6·2%, n=6). Re-exposure to Ca^{2+}-containing solution did not result in the development of spontaneous contraction, but refilling of the intracellular Ca^{2+} stores was observed, because a contraction was obtained in response to caffeine (Caf_2) (Table 2).

To study the possible action of tetrandrine and isotetrandrine on the refilling of the intracellular Ca^{2+} stores sensitive to caffeine, the magnitude of the contractile response obtained by subsequent addition of caffeine (Caf_2, Figure 4b) in Ca^{2+}-free medium after a Ca^{2+}-loading period was assumed to be related to the content of the agonist-sensitive Ca^{2+} pools. The recovery of the contractile response to caffeine in Ca^{2+}-free medium (Caf_2) was inhibited by tetrandrine but only at the highest concentration tested (300 μM) when it was present during the Ca^{2+}-refilling period; a lower concentration (100 μM) of alkaloid did not modify this contractile response. Isotetrandrine did not significantly modify the caffeine response (Caf_2) (Table 2).

Discussion

Tetrandrine has the pharmacological profile of a Ca^{2+}-entry blocker (Qian et al 1983; King et al 1988; D'Ocón et al 1992; Felix et al 1992; Wang & Lemos 1995), and its inhibition of the L-type Ca^{2+} channel has been considered to be the basis of its therapeutic efficacy in the treatment of cardiovascular disorders such as hypertension and angina (Gao et al 1965; Wang & Lemos 1995). The isomer of tetrandrine, isotetrandrine, also interacts at the benzothiazepine-binding site (Felix et al 1992) but with less potency than tetrandrine.

In a previous study Anselmi et al (1994) examined the relaxant action of tetrandrine and isotetrandrine in rat aortic strips in the presence and absence of extracellular calcium; the results indicated that both alkaloids relax, in a concentration–dependent manner, the contraction induced by noradrenaline and KCl and that inhibition of KCl-induced contraction by tetrandrine was stronger than that by isotetrandrine. In assays in Ca^{2+}-free solution, the highest concentration tested of both alkaloids inhibited the contractile response induced by noradrenaline; these results suggest that the alkaloids could inhibit contractile responses mediated by α_1-adrenoceptor activation. This hypothesis is consistent with the fact that in rat aorta the noradrenaline-induced release of intracellular Ca^{2+} is attributable to α_1-adrenoceptor activation (Noguera & D'Ocón 1992). To confirm this hypothesis we studied the possible interaction of tetrandrine and isotetrandrine with α_1-adrenoceptors using a radioligand-binding technique. Because of the limited amount of plasmalemma-rich membrane available from the rat aortic smooth muscle, rat cerebral cortex membranes were chosen for the radioligand-binding study. It has been demonstrated that in this preparation the prazosin-high-affinity sites are composed of α_{1A}- and α_{1B}-subtypes at a ratio of approximately 30:70 (Sallés & Badía 1994; Madrero et al 1996). Our results show that the alkaloids tested were unable to discriminate between α_1-adrenoceptor subtypes, because the binding of [^{3}H]prazosin was monophasically inhibited by these compounds with Hill slopes not significantly different from unity. The affinity for the two subtypes is, therefore, similar. These results provide us with experimental evidence that both alkaloids act at the α_1-adrenoceptor, but comparison of the K_i values obtained with [^{3}H]prazosin and [^{3}H]diltiazem (Felix et al 1992) reveals greater selectivity for interaction at benzothiazepine than at the α_1-adrenoceptor binding site.

Our functional studies showed that tetrandrine and isotetrandrine relax the contraction elicited by noradrenaline in physiological solution (Anselmi et al 1994) that depends on both extracellular Ca^{2+} influx through voltage Ca^{2+} channels and intracellular Ca^{2+} release by α_1-adrenoceptor activation (Noguera & D'Ocón 1996). The IC50 values obtained in these assays indicated higher potency compared with the IC50 values obtained for the contraction induced by noradrenaline in Ca^{2+}-free solution that is attributable only to intracellular Ca^{2+}-release by α_1-adrenoceptor activation (Table 1). This explains why both alkaloids have stronger inhibitory action on the entrance of extracellular calcium.

It is significant that the IC50 values obtained on noradrenaline-induced contraction in physiological

solution were very different from the K_i value obtained in binding studies by displacing [^{3}H]prazosin (Table 1). This difference might be explained by the different α_1-adrenoceptor subtypes in these tissues—rat cerebral cortex membranes are composed of α_{1A}- and α_{1B}-adrenoceptors (Sallés & Badía 1994; Madrero et al 1996), whereas α_{1D}-subtypes are present on the rat aorta (Kenny et al 1995; Fagura et al 1997). We can discount the possibility of agonist or partial agonist action (Achike & Kwan 1997) of the alkaloids, because results obtained in the current work showed that tetrandrine and isotetrandrine did not modify the basal tone of rat aorta or rat-tail artery (data not shown).

In another group of experiments we assayed the action of these compounds on the spontaneous contractile response elicited by extracellular calcium after depletion of noradrenaline-sensitive intracellular stores and on the refilling of intracellular Ca^{2+} stores sensitive to noradrenaline or caffeine. The increase in resting tone (IRT) seems to be related to α_1-adrenoceptor activation (it was selectively blocked by prazosin; Noguera & D'Ocón 1992) but the fact that calcium-channel blockers such as nifedipine (Noguera & D'Ocón 1993a), nimodipine (Noguera et al 1997) and verapamil (Noguera & D'Ocón 1993b) also block this contraction is indicative of the involvement of a voltage-dependent Ca^{2+} channel in this process. We assessed the influence of tetrandrine and isotetrandrine on the magnitude of this increase in the resting tone. Treatment with these agents induced concentration-dependent inhibition of the contractile response (IRT), because the IC50 values for tetrandrine and isotetrandrine were very similar to those obtained for contractile responses elicited by KCl (80 mM). We can assume that these alkaloids relaxed the IRT by blocking Ca^{2+} entry through voltage-sensitive Ca^{2+}-channels.

We also studied the possible action of tetrandrine and isotetrandrine on the Ca^{2+}-refilling process after depletion of intracellular stores sensitive to noradrenaline or caffeine. Noguera et al (1997) proposed the existence of two patterns of Ca^{2+} entry and refilling of internal stores, one involving voltage-dependent Ca^{2+}-channels sensitive to dihydropyridines and related to the IRT observed during this refilling, the other involving Ca^{2+} entry through a special refilling channel but apparently not related to the IRT. The mechanism involving Ca^{2+} entry through a special refilling channel is common to other agents that deplete internal Ca^{2+} stores, for example ryanodine, thapsigargin and caffeine (Noguera et al 1997) and is consistent with the capacitative Ca^{2+} entry model proposed by Putney (1990). In the current study we observed that the presence of tetrandrine and isotetrandrine during the refilling process resulted in inhibition of noradrenaline response (NA_3), and the IC50 values were similar to those obtained for contraction induced by KCl. We also observed that when, after depletion of Ca^{2+} stores by caffeine, the alkaloids were present during the refilling process there was no inhibition, and only tetrandrine at the highest concentration tested elicited significant inhibition of the caffeine response (Table 2). These results indicate that under our experimental conditions these alkaloids did not inhibit capacitative Ca^{2+} entry. These results contrast with those obtained by Low et al (1996) who considered tetrandrine a potent inhibitor of capacitative Ca^{2+} entry.

In summary, the results obtained in binding assays show that tetrandrine and isotetrandrine interact with α_1-adrenoceptors by displacing the [^{3}H]prazosin binding site and that tetrandrine interacts more selectively at the benzothiazepine recognition site in the Ca^{2+} channel (Felix et al 1992) than at the α_1-adrenoceptor binding site. The functional results indicate that both compounds mainly inhibit the Ca^{2+}-dependent process and have less action on α_1-adrenoceptors. The action of tetrandrine was always stronger than that of isotetrandrine.

Acknowledgements

This work was supported by Grant No. SAF 95-0538 from the Spanish Commision Interministerial de Ciencia y Tecnología. The authors thank Professor Dr D. Fang (Tongji Medical University, Wuhan, China) and Dr D. Cortes (University of València, Spain) for the donation of tetrandrine and isotetrandrine, respectively.

References

Achike, F. I., Kwan, C. Y. (1997) Tetrandrine induces α_1-adrenoceptor mediated contraction whereas phentolamine induced non-α-adrenoceptor-mediated relaxation of rat tail artery. Fed. Am. Soc. Exp. Biol. J. 11: 1248

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

Anselmi, E., Catret, M., Elorriaga, M., Ivorra, M. D. (1996) Interaction of tetrandrine and isotetrandrine with α_1-adrenoceptor. Methods Find. Exp. Clin. Pharmacol. 18 (Suppl. B): 176

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. Anal. Biochem. 72: 248–254

Cavé, A., Leboeuf, R., Hocquemiller, R., Bouquet, A., Fournet, A. (1979) Alcaloides de Limaciopsis loangensis. Planta Med. 35: 31–41

Cheng, Y. G., Prusoff, W. H. (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22: 3099–3108

D'Ocón, P., Blázquez, M. A., Bermejo, A., Anselmi, E. (1992) Tetrandrine and isotetrandrine, two bisbenzyltetrahydroisoquinoline alkaloids from Menispermaceae, with rat uterine smooth muscle relaxant activity. J Pharm. Pharmacol. 44: 579–582

Fagura, M. S., Lydford, S. J., Dougall, I. G. (1997) Pharmacological classification of α_1-adrenoceptors mediating contractions of rabbit isolated ear artery: comparison with rat isolated thoracic aorta. Br. J. Pharmacol. 120: 247–258

Felix, J. P., King, F., Shevell, J. L., García, M. L, Kaczorowski, G. J., Bick, I., Slaughter, R. S. (1992) Bis(benzylisoquinoline) analogs of tetrandrine block L-type calcium channels: evidence for interaction at the diltiazem-binding site. Biochemistry 31: 11793–11800

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

Gao, Y., Chang, M. Y., Mao, H. Y., Chao, H. Y., Chen, D. H. (1965) The clinical observation of tetrandrine in the treatment of 270 cases of hypertensive patients and hypertensive crisis. J. Chin. Intern. Med. 13: 504–597

Kenny, B. A., Chalmers, D. H., Philpott, P. C., Naylor, A. M. (1995) Characterization of an α_{1D}-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. Br. J. Pharmacol. 115: 981–986

King, V. F., García, M. L., Himmel, D., Reuben, J. P., Lam, Y. T., Pan, J., Han, G., Kaczorowski, G. J. (1988) Interaction of tetrandrine with slowly inactivating calcium channels. J. Biol. Chem. 263: 2238–2244

Low, A. M., Berdik, M., Sormaz, L., Gataiance, S., Buchanan, M. R., Kwan, C. Y., Daniel, E. E. (1996) Plant alkaloids, tetrandrine and hernandezine, inhibit calcium-depletion stimulated calcium entry in human and bovine endothelial cells. Life Sci. 58: 2327–2335

Madrero, Y., Elorriaga, M., Noguera, M. A., Cassels, B. K., D'Ocón, P., Ivorra, M. D. (1996) A possible structural determinant of selectivity of boldine and derivatives for the α_{1A}-adrenoceptor subtype. Br. J. Pharmacol. 119: 1563–1568

Munson, P. J., Rodbard, D. (1980) LIGAND: a versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. 107: 220–239

Noguera, M. A., D'Ocón, M. P. (1992) Different and common intracellular calcium-stores mobilized by noradrenaline and caffeine in vascular smooth muscle. Naunyn. Schmiedebergs Arch. Pharmacol. 345: 333–341

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

Noguera, M. A., D'Ocón, M. P. (1993b) Effects of different agents on the contractile response elicited by extracellular calcium after depletion of internal calcium stores in rat isolated aorta. J. Pharm. Pharmacol. 45: 701–706

Noguera, M. A., Ivorra, M. D., D'Ocón, P. (1996) Functional evidence of inverse agonism in vascular smooth muscle. Br. J. Pharmacol. 119: 158–164

Noguera, M. A., Ivorra, M. D., Chuliá, S., D'Ocón, P. (1997) Capacitative Ca^{2+} entry associated with α_1-adrenoceptors in rat aorta. Naunyn Schmiedebergs Arch. Pharmacol. 356: 83–89

Putney, J. W. (1990) Capacitative calcium-entry revisited. Cell Calcium 11: 611–634

Qian, J. Q., Thoolen, M. J. M. C., van Meel, J. C. A., Timmermans, P. B., van Zwieten, P. A. (1993) Hypotensive activity of tetrandrine in rats. Pharmacology 26: 187–197

Sallés, J., Badía, A. (1994) Selective enrichment with α_{1A}- and α_{1B}-adrenoceptor subtypes in rat brain cortical membranes. Eur. J. Pharmacol. 266: 301–308

Schiff, P. L. (1987) Bisbenzylisoquinoline alkaloids. J. Nat. Prod. 50: 529–599

Wang, G., Lemos, J. R. (1995) Tetrandrine: a new ligand to block voltage-dependent Ca^{2+} and Ca(+)-activated K^+ channels. Life Sci. 56: 295–306