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Tetrandrine and Isotetrandrine, Two Bisbenzyltetrahydroisoquinoline Alkaloids from *Menispermaceae*, with Rat Uterine Smooth Muscle Relaxant Activity

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Abstract—The effects of two bisbenzyltetrahydroisoquinoline alkaloids, 1*S*,1′*S* tetrandrine and its isomer 1*R*, 1′*S* isotetrandrine, were investigated in rat isolated uterus in order to identify the mechanism of relaxant action and to study the influence of the absolute configuration on the activity of these alkaloids. Both inhibited the uterine contraction induced by high K^+, acetylcholine and oxytocin. In Ca^{2+}-free medium, isotetrandrine relaxed the sustained contraction induced by oxytocin but tetrandrine did not. The relaxant effects of the alkaloids may be due to blockade of calcium influx through specific channels. Tetrandrine and isotetrandrine modify the calcium channel in a nonreversible manner whilst only isotetrandrine acts intracellularly. Tetrandrine shows a more specific relaxant activity as a calcium entry blocker.

Tetrandrine, a bisbenzyltetrahydroisoquinoline alkaloid isolated from the Chinese medicinal plant *Sephania tetrandra*, is used in China to treat angina and hypertension. Previous experiments have shown that tetrandrine exerts potent negative inotropic effects (Fang & Jiang 1986) in a variety of animal species, blocks the contraction of K^+-depolarized or noradrenaline-induced contraction in rabbit aorta, shifts the cumulative dose-response curves to the right and depresses the maximal response to $CaCl_2$ in uterine smooth muscle (Fang & Jiang 1986). These pharmacological studies suggest that this compound may function as a Ca^{2+} entry blocker but its precise mechanism of action remains unknown.

The chemical structure of tetrandrine (Fang & Jiang 1986) shows a dimer of two benzylisoquinoline subunits condensed in a head to head, tail to tail fashion with 1*S*, 1′*S* stereochemistry at the chiral isoquinoline carbons. Isotetrandrine alkaloid isolated from *Limaciopsis loangensis* (Cavé et al 1979) differs from tetrandrine only in the absolute stereochemistry at the chiral carbon atoms (Fig. 1).

In the present study we have examined the mechanism by which tetrandrine and its isomer, isotetrandrine, inhibit the contractile responses of rat uterine smooth muscle. Experiments in the presence or absence of extracellular calcium were performed to identify the mechanism of action of these alkaloids and to clarify the influence of the absolute configuration on its relaxant activity.

Materials and Methods

Preparation of uterine horns

Female Wistar rats, 150–200 g, were given oestradiol benzoate (5 mg kg^{-1}); 24 h later they were killed by a blow on the head and exsanguinated. One uterine horn was removed and mounted in a 10 mL organ-bath chamber filled with physiological solution bubbled with a mixture of 95% O_2–5% CO_2, 31°C.

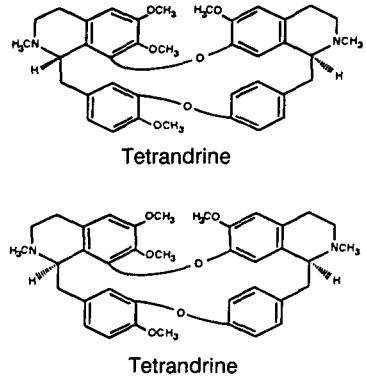

FIG. 1. Chemical structure of tendrandrine and isotetrandrine.

Experimental procedure

K^+-depolarized uterus. The organ was immersed in Jalón–Ringer solution and equilibrated for 20 min under a resting tension of 1 g and a prolonged tonic contraction was obtained (KCl_a). Two experimental designs were applied. In one series of experiments, three contractile responses to KCl were induced and alkaloids (10^{-4} M) were added 15 min before the second addition of depolarizing solution (KCl_b). After washing, another addition of depolarizing solution induced a contractile response (KCl_c). In another series, alkaloids were added in cumulative amounts (10 min interval between doses) when the second plateau of K^+-contraction (KCl_b) was reached (cumulative amounts 10^{-6}–$3{\cdot}33 \times 10^{-4}$ M). After washing, further addition of depolarizing solution induced a contractile response (KCl_c).

In order to investigate the effect of the alkaloids on β-adrenoceptors, this assay was run in the presence and absence of propranolol (10^{-5} M).

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Acetylcholine-induced contraction. A uterine horn was incubated in Jalón–Ringer solution with a resting tension of 1 g for 20 min. Acetylcholine (ACh, 10^{-4} M) was added, which induced an initial phasic contraction followed by a plateau with small rhythmic contractions. Two experimental procedures were performed as above. In the first, the alkaloids (10^{-4} M) were added 15 min before the second addition of acetylcholine (ACh_b) and after washing, a third contraction induced by this agonist was obtained (ACh_c). In the second, cumulative amounts of the alkaloids were added when the second contractile response to the agonist (ACh_b) was reached (cumulative amounts $10^{-6} - 3{\cdot}33 \times 10^{-4}$ M). After washing, another addition of acetylcholine induced a contractile response (ACh_c).

Oxytocin-induced rhythmic contractions. A uterine horn was incubated in Locke–Ringer solution with a resting tension of 1 g for 20 min. Oxytocin (0·01 unit mL^{-1}) was added, inducing rhythmic contractions. When these were stable the alkaloids were added cumulatively to the organ bath.

Oxytoxin-induced Ca^{2+}-free contractions. A uterine horn was equilibrated for 1 h in Locke–Ringer solutions under a resting tension of 0·5 g. The solution was then replaced by Ca^{2+}-free solution containing 3 mM EDTA and incubation was continued for 50 min. Subsequently, the solution was replaced by Ca^{2+}-free solution containing 1 mM EDTA and the uterus was incubated for 20 to 30 min. Sustained contractile responses to oxytocin (0·01 unit mL^{-1}) were obtained and cumulative amounts of alkaloids were added.

Isometric responses were measured using a recorder (Philips PM 8222) with an amplifier (8805C HP) and a force displacement transducer (Gould Statham UC2).

Solutions

The following solutions were used: Jalón–Ringer (mM): NaCl 154, KCl 5·63, $CaCl_2$ 0·648, $NaHCO_3$ 5·95 and glucose 2·77; depolarizing Jalon–Ringer (mM):NaCl 103·3, KCl 56·3 $CaCl_2$ 0·648, $NaHCO_3$ 5·95 and glucose 2·77; Locke–Ringer (mM): NaCl 154, KCl 5·63, $CaCl_2$ 2·16, $MgCl_2$ 2·10, $NaHCO_3$ 5·95 and glucose 5·55; Ca^{2+}-free Locke–Ringer: as for Locke–Ringer except for the omission of $CaCl_2$ and addition of EDTA 3 or 1 mM.

Drugs

Oxytocin and EDTA were purchased from Sigma Chemical Company. Tetrandrine was a generous gift from Dr Fang. Isotetrandrine was isolated by Cavé et al (1979). All drugs were dissolved in distilled water. All solutions were prepared daily and the pH 7 was confirmed. All other chemicals used were of analytical grade.

Statistical analysis

Relaxations are expressed as a percentage of the maximum tension obtained by agonist addition. E_{max} represents the maximal relaxation obtained after addition of the higher dose of each compound tested. A regression of response against $-\log C$ of test compound was performed by the least squares method for each preparation. The concentration needed to produce 50% inhibition (IC50) was obtained by linear regression of all points between 20–80% of the maximal response to the alkaloid tested.

Results are expressed as the mean ± s.e.m. of 5 or more preparations (n) obtained from different animals. Statistical significance of differences between the means was obtained using Student's *t*-test for unpaired data. *P*-values $< 0{\cdot}05$ were considered to represent significant differences.

Results

Relaxant effects of alkaloids on K^+-depolarized rat uterus

The three successive additions of KCl induced contractile responses of similar magnitude.

When alkaloids (10^{-4} M) were added 15 min before the second K^+-induced contraction (KCl_b), a lower contractile response was obtained. The phasic contraction to KCl in the presence of tetrandrine or isotetrandrine was significantly diminished in comparison with the contraction obtained when alkaloids were not present (Fig. 2A). The tonic component was completely inhibited. After washing, a new addition of KCl (KCl_c) did not reproduce the initial contraction.

The addition of tetrandrine and isotetrandrine when the plateau of the second K^+-induced contraction was reached produced concentration-dependent relaxation. Parameters of dose-response curves of relaxation are presented in Table 1. After washing the uterus, a new addition of KCl (56·3 mM) produced a contraction plateau (KCl_c) that was significantly different from the first.

In the presence of propranolol (10^{-5} M), the alkaloids completely relaxed the KCl-induced contraction and the IC50 was not different from that obtained in the absence of propranolol (IC50 of tetrandrine: 43·8 ± 13·3 μM, n = 5; IC50 of isotetrandrine: 45·3 ± 5·6 μM, n = 5). This concentration of

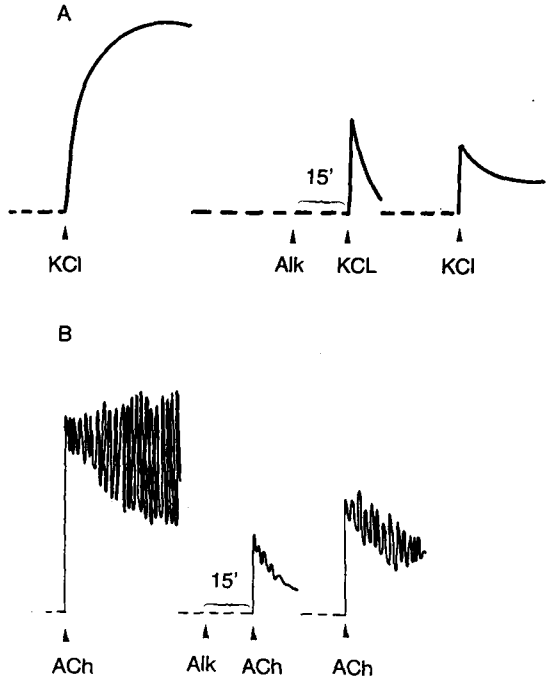

FIG. 2. Effect of alkaloids (10^{-4} M) added 15 min before the second addition of (A) depolarizing solution and (B) acetylcholine (10^{-4} M).

Table 1. Parameters of dose-response curves for the relaxation induced by cumulative doses of tetrandrine and isotetrandrine in rat KCl-depolarized uterus, acetylcholine (ACh) contraction, oxytocin ($OT_{Ca(+)}$) (0·01 units mL^{-1}) contractions and oxytocin ($OT_{Ca(-)}$) (0·01 unit mL^{-1}) Ca^{2+}-free contractions.

	Tetrandrine			Isotetrandrine		
	E_{max} (%)	IC50 (10^{-5} M)	n	E_{max} (%)	IC50 (10^{-5} M)	n
KCl	100·5 ± 1·0	3·2 ± 0·5	5	106·3 ± 4·2	2·7 ± 0·3	5
ACh	100·1 ± 1·3	2·8 ± 0·5	9	91·7 ± 1·4	3·9 ± 0·9	5
$OT_{Ca(+)}$	94·5 ± 3·4	4·5 ± 0·9	6	83·8 ± 4·2	4·9 ± 1·5	4
$OT_{Ca(-)}$				89·1 ± 2·2	3·5 ± 1·1	6

Values are mean ± s.e.m.

propranolol has previously been shown to block the relaxant action of isoprenaline.

Relaxant effects of alkaloids on acetylcholine-induced contraction of rat uterus

When the uterus was preloaded with tetrandrine or isotetrandrine (10^{-4} M), 15 min before the addition of acetylcholine (10^{-4} M) (ACh_b), inhibition of the phasic peak contraction with respect to the contractile response obtained in the absence of the alkaloids was observed. The rhythmic contractions were abolished only by tetrandrine (Fig. 2B). After washing the uterus, a new addition of acetylcholine (10^{-4} M) (ACh_c) did not reproduce the first contraction.

Addition of tetrandrine and isotetrandrine during the second plateau of contraction by acetylcholine (ACh_b) produced dose-dependent relaxations; hence, dose-response (relaxation) curves were constructed by addition of cumulative doses of alkaloids (Table 1). After washing the uterus, a new addition of acetylcholine (ACh_c) (10^{-4} M) produced a contraction that was significantly different from the first.

Modification of uterine response to oxytocin by the alkaloids

The addition of oxytocin (10^{-2} units mL^{-1}) to the uterine horn incubated in Locke–Ringer solution induced rhythmic contractile responses of stable frequency and amplitude (3668·40 ± 447·32 mg, n = 5). The addition of cumulative concentrations (10^{-6}–$3·33 \times 10^{-4}$ M) of both alkaloids diminished the frequency and amplitude of the contraction in a concentration-dependent manner. IC50 values obtained for each alkaloid were similar to those obtained for experiments with acetylcholine of KCl (Table 1).

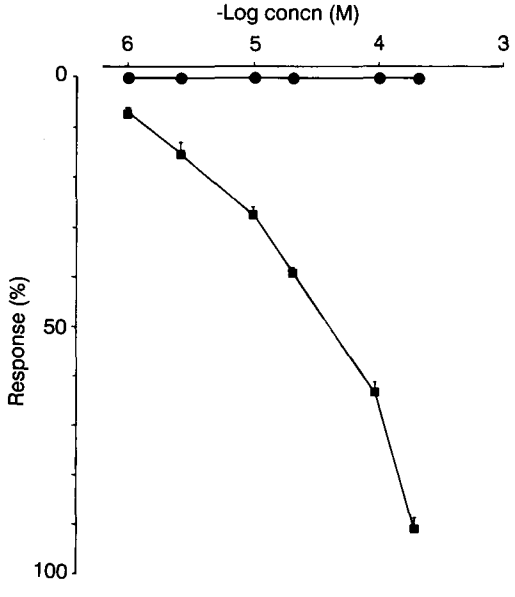

FIG. 3. Dose response curves of relaxation to alkaloids in uterus previously contracted with oxytocin (0·01 units mL^{-1}) incubated in Ca^{2+}-free EDTA-containing solution. ● Tetrandrine (n = 5), ■ isotetrandrine (n = 5). Bars represent s.e. of n experiments.

Effects of alkaloids on contractile response of uterus to oxytocin in Ca^{2+}-free medium

The sustained contractions (343·0 ± 1·7 mg, n = 5) induced by oxytocin (0·01 units mL^{-1}) after 20 min in Ca^{2+}-free EDTA-containing solution were not modified when cumulative doses (10^{-6}–$3·33 \times 10^{-4}$ M) of tetrandrine were added (n = 5) (Fig. 3). In contrast, cumulative doses (10^{-6}–$3·33 \times 10^{-4}$ M) of isotetrandrine produced concentration-dependent relaxation (Table 1).

Discussion

The results show that tetrandrine and its isomer, isotetrandrine, are able to inhibit several models of agonist-induced contractions in smooth muscle.

In the presence of extracellular Ca^{2+}, both alkaloids inhibited, in a concentration-dependent manner, the contractile response induced by depolarization with acetylcholine or oxytocin, when they were added before or during the contractile plateau elicited by each agonist. Examination of characteristic parameters of dose-response curves of relaxation indicates that E_{max} was similar in all cases and IC50 was not significantly different.

The relaxant action exhibited by tetrandrine and isotetrandrine was similar in the presence or absence of propranolol. This indicates that the relaxation induced by the alkaloids cannot be attributed to a β-stimulating action. These results agree with those of previous studies with tetrandrine in rabbit aorta and coronary arteries (Fang & Jiang 1986).

The response to KCl includes a biphasic contraction consisting of an early phasic component and delayed tonic component that is the result of increased Ca^{2+} influx through voltage-sensitive calcium channels (Godfraind & Kaba 1972). The phasic contraction was lower in the presence of the alkaloids tested but the tonic contraction was abolished when the uterus was preincubated with the alkaloid or when cumulative concentrations of tetrandrine or isotetrandrine were added after the sustained contractile plateau induced by KCl was reached. It is interesting to note that contractile

responses to KCl after removal of the alkaloid from the medium did not recover in any of the experimental procedures. These results indicate that the alkaloids affect KCl-sensitive Ca^{2+}-channels by a mechanism that is not reversible by washing.

The mechanism underlying the rise in cytoplasmic Ca^{2+} induced by acetylcholine and oxytocin is complex and probably involves both voltage-dependent and receptor-operated calcium channels (Bolton 1979; Edwards et al 1986; Savineau & Mironneau 1990), as well as release of intracellular Ca^{2+} ions (Villar et al 1986; Anselmi et al 1987; D'Ocón et al 1987a, b). Tetrandrine and isotetrandrine relax uterine contraction induced by both agonists in a dose-dependent manner. When alkaloids were added before the second addition of acetylcholine a lower but sustained contractile response was obtained, unlike KCl-induced contraction in the presence of the alkaloids. These results suggest a more specific action on the contractile response induced by KCl.

In order to determine the possible action of alkaloids on intracellular Ca^{2+}, experiments were designed in Ca^{2+}-free medium. Under these conditions only oxytocin can promote a sustained contraction (Anselmi et al 1987; D'Ocón et al 1987a). Acetylcholine induces a slight phasic contraction (D'Ocón et al 1987b) and KCl does not elicit uterine contractile responses (Villar et al 1986). In Ca^{2+}-free EDTA-containing solution the maintained contraction evoked by oxytocin is mediated by the release of Ca^{2+} from intracellular calcium stores through activation of a specific receptor (Anselmi et al 1987). The present results indicate that tetrandrine has no effect on the release of Ca^{2+} from an intracellular store or stores sensitive to oxytocin but since isotetrandrine relaxed oxytocin contractions in Ca^{2+}-free solution in a dose dependent manner, it may act intracellularly.

The different behaviour of the two alkaloids on uterine contraction promoted by the release of intracellular Ca^{2+} may be related to the absolute configuration at the chiral centres. The alkaloid with absolute configuration 1*S*, 1′*S* (tetrandrine) exerts a selective inhibitory action on the calcium influx from the extracellular medium without changing the intracellular distribution of this ion. The alkaloid with absolute configuration 1*R*, 1′*S* (isotetrandrine) has a non-specific effect on extracellular influx and intracellular calcium levels. These observations suggest the existence of a stereospecific receptor site at the intracellular level; similar observations were made in previous studies on the antioquine series and its derivatives (D'Ocón et al 1989; Ivorra et al, 1992) or papaverine and its derivatives (Anselmi et al, 1992). When these same experimental procedures are applied using nifedipine as a relaxant of uterine contraction, the results resemble those obtained with tetrandrine (D'Ocón et al 1991).

These results confirm those of King et al (1988) who showed that tetrandrine interacts directly at the benzothiazepine-binding site of the Ca^{2+}-channel and modulates ligand binding allosterically at other receptors in this complex. More recently, Liu et al (1991) demonstrated that tetrandrine is a calcium channel antagonist which inhibits both T and L channel currents in ventricular cells.

The present findings suggest that the relaxant effects of tetrandrine and isotetrandrine may be due to blockade of calcium movements across the cell membrane through specific channels. Tetrandrine and its isomer, isotetrandrine, modify the calcium channel activity in a permanent manner but only isotetrandrine acts intracellularly. Tetrandrine shows a more specific relaxant activity and inhibits calcium entry.

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