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COMMUNICATIONS

Comparative study of the rat uterine smooth muscle relaxant activity of three bisbenzyltetrahydroisoquinolines with tetrandrine

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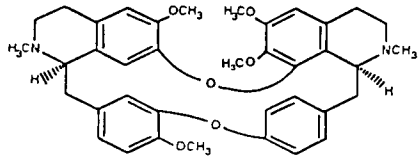
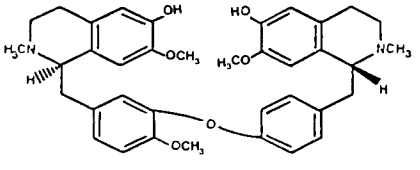
Abstract—The relaxant activity of three bisbenzyltetrahydroisoquinolines—obaberine, popisonine and lindoldhamine—was examined in rat isolated uterus and their inhibitory potencies were compared with that of tetrandrine. All alkaloids tested relaxed KCl-depolarized rat uterus and totally or partially inhibited oxytocin-induced rhythmic contractions. The degree of methylation of the free phenolic hydroxy groups and the loss of one diarylether bridge influence the potency of relaxant action of these alkaloids. Only alkaloids with absolute configuration 1*R*,1′*S* or 1*R*1′*R* acted intracellularly, promoting relaxation of contractile responses induced by oxytocin or vanadate in a Ca^{2+}-free medium.

As a continuation of our attempt to clarify the action of bisbenzylisoquinoline alkaloids (BBIQ) on smooth muscle contraction, we examined the mechanism of the inhibitory action of obaberine, popisonine and lindoldhamine on rat isolated uterus in comparison with that of tetrandrine, the main alkaloid isolated from the stem bark of *Stephania tetrandra* and considered a calcium antagonist (Wang & Liu 1985; Fang & Jiang 1986a, b; Yao et al 1987; Triggle et al 1989; D'Ocón et al 1992).



Obaberine



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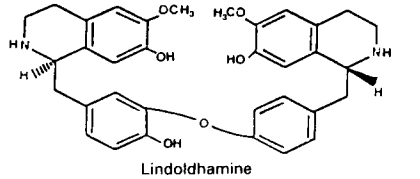
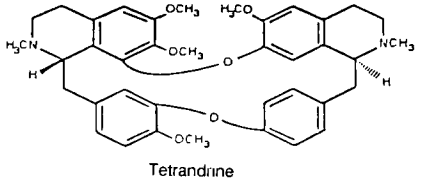


Lindoldhamine



Tetrandrine

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Obaberine, like tetrandrine, is a dimer of two benzylisoquinoline subunits condensed in a head to head (7,8′), tail to tail (11,12′) fashion but with 1*R*,1′*S* stereochemistry at the chiral isoquinoline carbons; popisonine and lindoldhamine are constituted by a single diarylether bridge (11,12′) and both present an absolute configuration 1*R*,1′R.

The aim of the present work was to determine the influence of the absolute configuration, the presence of one of two diarylether linkages between the benzylisoquinoline subunits and the influence of *N*-methylation on the isoquinoline rings.

Material 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 filled with physiological solution bubbled with 95% O_2–5% CO_2 at 31°C.

Experimental procedure. K^+-depolarized uterus. The organ was immersed in Jalon–Ringer solution and equilibrated for 20 min under a resting tension of 1 g. Tissues were then exposed to depolarizing solution (KCl 56·3 mM), which induced a rapid contraction, followed by a slight relaxation and then a prolonged contraction. During this plateau phase of contraction, cumulative doses of alkaloids were administered and dose-related relaxations could be observed. After washing, further addition of depolarizing solution induced a contractile response.

To avoid a possible influence of the alkaloids on the release of catecholamines or any effect on β-adrenoceptors, we ran this assay in the presence and in the absence of propranolol (10^{-5} M).

Oxytocin-induced rhythmic contractions. The uterine horn was incubated in Locke–Ringer solution under a resting tension of 1 g for 20 min. Rhythmic contractions were induced by addition of oxytocin (0·01 unit mL^{-1}). The alkaloids were then added cumulatively to the organ bath.

Oxytocin- or vanadate-induced Ca^{2+}-free contraction. Uterine horns were equilibrated for 1 h in the Locke–Ringer solution 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 20–30 min. Sustained contractile response to oxytocin (0·01 units mL^{-1}) or vanadate (100 μM) was obtained and cumulative amounts of alkaloids were added.

Isometric responses were measured using a recorder (Philips PM 8222) with an amplifier (8850C HP) and a force displacement transducer (Gould Statham UC2).

Solutions. The following solutions were used: Jalon–Ringer solution (mM): NaCl 154, KCl 5·63, $CaCl_2$ 0·648, $NaHCO_3$ 5·95 and glucose 2·77; depolarizing solution (mM): NaCl 103·3, KCl 56·3, $CaCl_2$ 0·648, $NaHCO_3$ 5·95 and glucose 2·77; Locke–Ringer solution (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 solution: the same composition as the Locke–Ringer solution but with $CaCl_2$ omitted and EDTA (3 or 1 mM) added.

Drugs and chemicals. Oxytocin, vanadate and EDTA were purchased from Sigma Chemical Company (USA). Tetrandrine (*Stephania tetrandra*) was a generous gift from Dr Fang. Obaberine (*Pseudoxandra sclerocarpa*) was isolated by Cortes et al (1985). Popisonine (*Popowia psisocarpa*) and lindoldhamine (*Polyalthia nitidissima*) were isolated by Jossang et al (1983, 1986).

All other chemicals used were of analytical grade. All drugs were dissolved in distilled water, prepared daily and the pH was measured.

Statistical analysis. Relaxation was expressed as a percentage of the maximum tension obtained by agonist addition. E_{max} represents the maximal relaxation obtained after addition of the highest 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 and 80% of the maximal response to the alkaloid tested.

Results are expressed as the mean ± s.e.m. of five or more preparations (n) obtained from different animals. Statistical significance of differences between the means was assessed using Student's *t*-test for unpaired data. *P* values of less than 0·05 were considered to represent significant differences.

Results

Relaxant effects of alkaloids tested on rat K^+-depolarized uterus. The addition of tetrandrine, obaberine, popisonine or lindoldhamine when the plateau of K^+-induced contraction was reached produced dose-dependent relaxations; hence, dose-response (relaxation) curves were constructed by addition of cumulative doses of alkaloids (10^{-6}–3×10^{-4} M). Table 1 summarizes the maximal relaxation (E_{max}) and IC50 for each product tested. The relaxant activity shown by obaberine was similar to that shown by tetrandrine, but popisonine and lindoldhamine exhibited less potency. After washing the uterus a new addition of KCl (56·3 mM) produced a contraction plateau that was significantly different from the first.

In another set of experiments preincubation with propranolol (10^{-5} M) made no significant difference to E_{max} and IC50 values observed after addition of alkaloids in cumulative amounts. The concentration of propranolol used was that previously shown to block the relaxant action of isoprenaline.

Table 2. Parameters of dose-response relaxation curves obtained after addition of cumulative amounts of alkaloids in rat uterus previously contracted by oxytocin (0·01 units mL^{-1}).

	E_{max} (%)	IC50 (μM)	n
Tetrandrine	94·5 ± 3·4	45·3 ± 9·0	6
Obaberine	50·0 ± 4·2	—	5
Popisonine	101·3 ± ·07	33·9 ± 1·6	3
Lindoldhamine	101·4 ± 0·5	29·4 ± 1·5	5

Values are mean ± s.e.m.

Modification of uterine response to oxytocin by alkaloids. Addition of 0·01 units mL^{-1} oxytocin to the uterine horn incubated in Krebs–Henseleit solution induced a rhythmic contractile response with stable frequency and amplitude. The addition of cumulative doses (10^{-6}–3×10^{-4} M) of alkaloids diminished both the frequency and the amplitude of the contraction in a dose-dependent manner. Table 2 summarizes the E_{max} and IC50 for each product tested.

The largest doses tested for tetrandrine, popisonine and lindoldhamine (3×10^{-4} M) abolished contractile responses to oxytocin, but at the same dose obaberine showed much lower relaxant activity; therefore, an IC50 value for this alkaloid could not be calculated. After washing, complete recovery of rhythmic contraction induced by a new addition of oxytocin (0·01 units mL^{-1}) was observed.

Effects of alkaloids on the contractile response of uterus to oxytocin in Ca^{2+}-free medium. Oxytocin (0·01 units mL^{-1}) applied for a longer period (15 min) induced sustained uterine contractions beyond 20 min in Ca^{2+}-free medium; the amplitude of these contractions was 354·3 ± 27·5 mg (n = 17). When obaberine was added in cumulative doses (10^{-6}–3×10^{-4} M) to the Ca^{2}-free solution during the plateau phase of this contraction, complete relaxation was observed. In contrast, only partial relaxation occurred when cumulative doses of popisonine or lindoldhamine were added and tetrandrine showed no effect. The maximal relaxation and IC50 for each alkaloid are summarized in Table 3.

Effects of alkaloids on vanadate-induced Ca^{2+}-free contraction. Vanadate (100 μM) induced a sustained response as long as the uterus was exposed to the agonist and the amplitude of this contraction was 521·6 ± 72·8 mg (n = 17). When alkaloids were added in cumulative amounts (10^{-6}–3×10^{-4} M), dose-dependent relaxations were obtained. When cumulative doses of popisonine or lindoldhamine were added, only partial relaxation occurred and tetrandrine showed no effect. The maximal relaxation and IC50 values for each alkaloid are summarized in Table 3.

Table 1. Parameters of dose-response curves of relaxation induced by cumulative doses of alkaloids on rat KCl-depolarized uterus in presence and absence of propranolol (10^{-5} M).

	Without propranolol			With propranolol		
	E_{max} (%)	IC50 (μM)	n	E_{max} (%)	IC50 (μM)	n
Tetrandrine	100·5 ± 1·0	32·4 ± 5·9	5	95·1 ± 4·1	43·8 ± 13·3	5
Obaberine	100·0 ± 3·7	22·3 ± 7·4	5	95·7 ± 3·0	37·6 ± 8·3	5
Popisonine	95·1 ± 2·9	53·6 ± 5·7*	5	90·8 ± 2·2	61·2 ± 2·9	5
Lindoldhamine	97·4 ± 1·3	54·6 ± 10·3*	4	92·6 ± 1·0	51·5 ± 6·7	5

*$P < 0·05$ vs tetrandrine. Values are mean ± s.e.m.

Table 3. Parameters of dose-response curves obtained after cumulative addition of different alkaloids in uterus previously contracted by oxytocin (0·01 units mL^{-1}) or vanadate (100 μM) in Ca^{2+}-free EDTA-containing solution.

	Oxytocin (0·01 units mL^{-1})			Vanadate (100 μM)		
	E_{max} (%)	IC50 (μM)	n	E_{max} (%)	IC50 (μM)	n
Obaberine	100·1 ± 3·5	12·10 ± 2·3	5	93·9 ± 3·0	29·9 ± 6·3	6
Popisonine	69·9 ± 4·5	110·6 ± 17·8*	6	64·8 ± 3·3	221·4 ± 63·0*	5
Lindoldhamine	76·8 ± 4·7	56·5 ± 15·6*	5	55·2 ± 2·3	—	6

*$P < 0·05$ vs obaberine. Values are mean ± s.e.m.

Discussion

The present results show that the alkaloids studied were able to inhibit several models of agonist-induced contractions in uterine smooth muscle. Some models involve depolarization elicited by high concentrations of KCl which induce Ca^{2+}-entry by voltage-operated channels (VOCs) (Bolton 1979; Amedée et al 1986; Ballejo et al 1986; Edwards et al 1986; Granger et al 1986); another model includes oxytocin contractions that involve a more complex mechanism related to release of intracellular Ca^{2+} (Villar et al 1985; Anselmi et al 1987; D'Ocón et al 1987, 1989) and Ca^{2+}-entry from the extracellular space (Edwards et al 1986; D'Ocón et al 1989). In order to determine the possible action of alkaloids on the intracellular Ca^{2+} level, we designed experiments in Ca^{2+}-free medium. The contractile response induced by oxytocin in Ca^{2+}-free medium is related exclusively to the release of Ca^{2+} from the intracellular stores (Carsten & Jordan 1987; Anselmi et al 1987; D'Ocón et al 1989). The sustained contraction induced by vanadate in Ca^{2+}-free solution can also be related to release of Ca^{2+} from intracellular storage sites (D'Ocón 1989) and this action can be explained by the results of previous studies showing vanadate as a potent inhibitor of Ca^{2+}-Mg ATPase (Mironneau et al 1984; Nechay 1984) and Na-K ATPase (Grover et al 1981).

Our results show that when extracellular calcium is present, obaberine inhibits sustained K^+-induced contraction with the same potency as tetrandrine, but the rhythmic contraction induced by oxytocin was diminished to a much lesser extent. This behaviour indicates that the relaxant mechanism of obaberine may be mainly related to Ca^{2+} influx, possibly through VOCs. Popisonine and lindoldhamine relax KCl contractile response and rhythmic contraction elicited by oxytocin, but both alkaloids showed a more potent relaxant effect on oxytocin-induced contraction. We can also see that both alkaloids have a less potent relaxant action on KCl contractile response than does tetrandrine. We have demonstrated that the methylation of the free phenolic hydroxylated compounds that showed an enhanced activity to the parent alkaloid, antioquine (Ivorra et al 1992).

Testing the effect of the alkaloids on KCl-depolarized rat uterus in presence of a β-blocker agent (propranolol) demonstrated that the relaxant action exhibited by the alkaloids was similar in the presence and in the absence of this agent, suggesting that the relaxation induced by the alkaloids cannot be attributed to a β-stimulating action.

In Ca^{2+}-free medium, obaberine, popisonine and lindoldhamine relaxed the uterine contraction induced by oxytocin and vanadate, while tetrandrine did not exhibit any relaxant activity. These results are in agreement with those of previous research that demonstrated that compounds with the absolute configuration 1*S*,1′*S*, such as tetrandrine (D'Ocón et al 1992), or 1*S*,1′*R*, such as antioquine (Ivorra et al 1992), did not exhibit any relaxant activity on sustained contraction induced by oxytocin, and this excludes a possible intracellular action. In contrast, alkaloids with the absolute configuration 1*R*,1′*S* such as isotetrandrine (D'Ocòn et al 1992), monterine and granjine (1*R*,1′*S*) (Ivorra et al 1992), relax uterine contraction elicited by oxytocin in Ca^{2+}-free medium.

The results obtained in our experimental conditions indicate that obaberine, popisonine and lindoldhamine have an intracellular site of action. It may be that these alkaloids interact with 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) and papaverine and its derivatives (Anselmi et al 1992). It is interesting to note that popisonine and lindoldhamine elicited a less potent relaxant response in oxytocin- or vanadate-induced contractions compared with obaberine, and it is possible that the reason for this diminished potency is the loss of the diarylether bridge (8,7′) or the minor degree of methylation of the free phenolic hydroxy groups.

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The effect of therapeutic doses of paracetamol on liver function in the rat perfused liver

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Abstract—The isolated liver perfusion technique was used to study the effect of therapeutic doses of paracetamol on hepatic gluconeogenesis and bromosulphthalein clearance from the perfusate and biliary excretion of the dye in the rat. Six groups of rats were studied; those in the three experimental groups were given 0·02 g kg^{-1} paracetamol daily for ninety days. The livers of animals in the control group and in one of the experimental groups were perfused with a medium containing pyruvate. The animals in the second experimental and control group were perfused with a medium containing bromosulphthalein (10 mg/100 mL). The livers of the third experimental and control group were subjected to histological examination. The rate of glucose formation and glucose concentrations were decreased, while, lactate levels and lactate: pyruvate ratios were increased in paracetamol-treated rats. The mean concentration of bromosulphthalein in the perfusate and biliary excretion of the dye were decreased. Macro and micro vesicular fatty change was present in the livers of paracetamol-treated rats. This study demonstrates that chronic administration of therapeutic doses of paracetamol to rats adversely affects liver function, as evidenced by impaired gluconeogenesis and bromosulphthalein clearance from the perfusate, and excretion of the dye into the bile, and provides histological evidence of hepatic damage in rats.

Paracetamol, a medication available over the counter without a prescription, is a major component of many formulations available for the relief of headaches, fever, coughs and cold, and it is the most widely used analgesic/antipyretic agent because of its overall efficacy and safety. In contrast to aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) it does not cause gastrointestinal bleeding, and there is no association of its use with the development of Reye's syndrome. The major problem caused by paracetamol is hepatotoxicity, which may be fatal after large doses of the drug (Black 1984; Hall et al 1986 a, b; O'Dell et al 1986; Monteagudo & Folb 1987). In-vivo studies of glucose metabolism and bromosulphthalein clearance have shown that impaired gluconeogenesis and bromosulphthalein clearance often occurs after liver damage due to paracetamol overdose (Davis et al 1975; Record et al 1975).

Earlier studies have shown that in chronic alcoholics, paracetamol hepatotoxicity occurs with therapeutic or near therapeutic doses of paracetamol (McLain et al 1980; Johnson et al 1981; Seeff et al 1986). A recent report showed that in two patients with a high daily alcohol consumption, liver damage exhibiting clinical and morphological features of paracetamol hepatotoxicity occurred; although the patients took only therapeutic doses of paracetamol (Floren et al 1987). This study shows that the isolated perfusion model has proved to be suitable and useful in providing information on the effects of therapeutic doses of paracetamol on liver function (gluconeogenesis and bromosulphthalein clearance) in livers not damaged by alcohol.

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Materials and methods

The protocol was approved by the Ethics Committee, University of Natal Medical School. The study conformed with accepted principles in the care and use of animals for experimental purposes set out by The Medical Research Council, South Africa.

Sixty six male Wistar rats, 200–220 g, of the University of Natal inbred strain, were kept in stainless steel cages with plastic floors. Each rat was individually earmarked and randomly assigned to one of six groups, three control and three experimental, with eleven rats per group. All the animals were fed on a rat standard diet. Water was freely available. The animals in the experimental groups were given 0·02 g kg^{-1} paracetamol and the control group was given an equal amount of distilled water by