J. Pharm. Pharmacol. 1992, 44: 337–343
Received July 17, 1991

Selective Inhibition of Calcium Entry Induced by Benzylisoquinolines in Rat Smooth Muscle

ELSA ANSELMI, GLORIA FAYOS, RAFAEL BLASCO, LUZ CANDENAS, DIEGO CORTES AND PILAR D'OCON

Laboratorio de Farmacognosia y Farmacodinamia, Departamento de Farmacologia, Facultad de Farmacia, Universidad de Valencia, 46010 Valencia, Spain

Abstract—The mechanism of relaxant activity of six benzylisoquinolines was examined in order to determine the minimal structural requirements that enable these compounds to have either a non-specific action like papaverine or an inhibitory activity on calcium entry via potential-operated channels. All the alkaloids tested totally or partially relaxed KCl-depolarized rat uterus and inhibited oxytocin-induced rhythmic contractions. Only glaucine and laudanosine inhibited K^+-induced uterine contractions more than oxytocin-induced uterine contractions. In Ca^+-free medium, sustained contractions induced by oxytocin or vanadate were relaxed by the alkaloids tested except for glaucine and laudanosine indicating no inhibitory effect on intracellular calcium release. Those alkaloids containing an unsaturated heterocyclic ring (papaverine, papaverinol, papaveraldine, *N*-methylpapaverine and dehydropapaverine) exhibited a more specific activity than those with a tetrahydroisoquinoline ring.

Calcium entry blockers comprise a chemically heterogeneous group of compounds that act primarily through interactions with specific sites at the L-class of voltage-operated calcium channels. These calcium channel blockers play a critical role in regulating the activity of smooth muscle and reducing Ca^{2+} entry through potential-operated calcium channels (POCs), thereby promoting an inhibition of smooth muscle tension (Godfraind et al 1986; Triggle et al 1989).

The major structural categories of compounds active at POCs are phenylalkylamines (related to papaverine), 1,4 dihydropyridines and benzothiazepines. Furthermore, several new structural classes of ligands reported recently appear to exert their effects primarily at the L-type of calcium channel. One of these, tetrandrine, a bis-benzylisoquinoline alkaloid, is an allosteric modulator of phenylalkylamine and 1,4 dihydropyridine binding that apparently operates through interactions at the benzothiazepine receptor of the L-channel (King et al 1988; Triggle et al 1989). Another bis-benzylisoquinoline, antioquine, has shown a selective smooth muscle relaxant activity through blockade of Ca^{2+}-movements across the cell membrane, mainly diminishing the Ca^{2+} entry via POCs (D'Ocon et al 1989).

Similar results were obtained in the benzylisoquinoline series, and compounds such as cularines, isocrasifoline (D'Ocon et al 1991) and aporfines (Cortes et al 1990) show a mechanism of smooth muscle relaxant activity which is not similar to papaverine in spite of their structural relationship. Papaverine is a non-specific smooth muscle relaxant and the molecular mechanism of its action is not yet known (Bolton 1979; Cumiskey & Feigenson 1983; D'Ocon 1989), whereas the alkaloids mentioned above have a more specific activity, closely related to the inhibition of Ca^{2+} entry via POCs, observed, for example, with nifedipine (D'Ocon et al 1991).

In the present study we have examined the mechanism of the inhibitory action of papaverine derivatives on uterine smooth muscle in the presence or absence of extracellular calcium, in order to determine the minimal structural requirements that enable benzylisoquinolines to have both a non-specific effect—like papaverine—on the entry or distribution of Ca^{2+} in the cell, and a specific inhibitory effect on Ca^{2+} entry via POCs shown by other benzylisoquinolines and closely related to the action of nifedipine.

Correspondence: E. Anselmi, Laboratorio de Farmacognosia y Farmacodinamia, Departamento de Farmacologia, Facultad de Farmacia, Universidad de Valencia, Avda. Blasco Ibañez 13, 46010 Valencia, Spain.

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 filled with Jalon-Ringer solution bubbled with a mixture of 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 with a resting tension of 1 g. A depolarizing solution was added (KCl 56·3 mM) and this addition caused a rapid contraction, followed by a slight relaxation and a prolonged contraction. In these experimental conditions, cumulative doses of alkaloids were administered and dose-related relaxations could be observed. After washing, further addition of depolarizing solution induced a contractile response.

In order to avoid a possible influence of the alkaloids on the release of cathecholamines or any effect on β-adrenoceptors, the assay was carried out in the presence and absence of propranolol (10^{-5} M).

Oxytocin-induced rhythmic contractions. The 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 and rhythmic contractions were induced by this agonist. Cumulative amounts of alkaloids were added to the organ bath.

Oxytocin- or vanadate-induced Ca^{2+}-free contraction. The uterine horn was equilibrated for 1 h in Locke-Ringer solution under a resting tension of 0·5 g. The solution was 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 responses to vanadate (100 μM) were obtained and cumulative amounts of alkaloids were added.

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

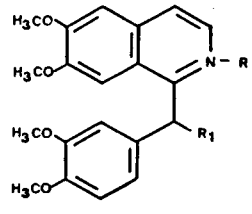
Solutions

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-free solution; the same composition as the Locke-Ringer solution with the omission of $CaCl_2$ and the addition of EDTA 3 or 1 mM.

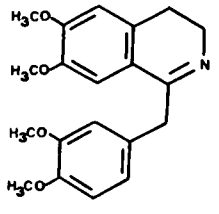
Drugs and chemicals

The structures of drugs studied are shown in Fig. 1.

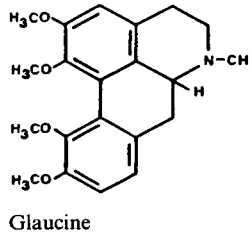
Oxytocin, vanadate, papaverine, (±)-laudanosine, (−)-glaucine and EDTA were purchased from Sigma Chemical Company. All solutions were prepared daily and the pH tested. All other chemicals used were of analytical grade.



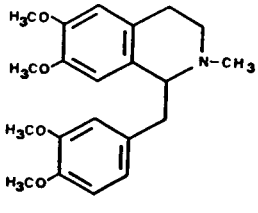
Benzylisoquinolines	R	R_1
Papaverine	—	H
N-Methylpapaverine	CH_3	H
Papaverinol	—	OH
Papaveraldine	—	=O



Dihydropapaverine



Glaucine



Laudanosine

FIG. 1. Chemical structures of papaverine, *N*-methyl papaverine, papaverinol, papaveraldine, dihydropapaverine, glaucine and laudanosine.

Preparation of papaverine derivatives

3,4 Dihydropapaverine. 2′,3′-Dimethoxyphenylacetyl chloride was obtained in quantitative yield by refluxing 2′,3′-dimethoxyphenylacetic acid (2·72 g) with 3·4 mL (46 mM) of thionyl chloride for 24 h. A mixture of 8·58 g (40 mM) of (2′,3′-dimethoxyphenyl)ethanoyl chloride and 13·5 mL (80 mM) of 2,3-dimethoxyphenylethylamine was heated under reflux for 12 h, to yield the corresponding amide. 3,4-Dihydropapaverine was obtained by treating 5 g (12·66 mM) of the amide with 5·5 g (26·4 mM) of phosphorus pentachloride. This mixture was stirred and heated under reflux for 12 h. Ethanol (30 mL) was added to remove the phosphorus oxychloride formed during the reaction. A mixture of ether/water (500 mL) was added and the resulting solution alkalinized by addition of sodium hydroxide. The 3,4-dehydropapaverine obtained (8·86 mM) was extracted with chloroform, recrystallized from methanol and converted into its hydrochloride for pharmacological testing.

Papaveraldine. Papaverine 1·770 g (5·22 mM) was allowed to react with potassium permanganate 0·332 g (2·10 mM) in acetone for 1 h at room temperature (21°C) with stirring. The reaction mixture was filtered and the liquid phase evaporated. The residue was washed and dissolved in methylene dichloride. Evaporation of solvent gave 1·324 g (72%) of the product.

Papaverinol. Papaveraldine 0·438 g (1·24 mM) was treated with sodium borohydride 1·80 g (1·24 mM) in methylene dichloride/methanol solution for 12 h. The resulting mixture was washed with water and extracted with methylene dichloride. Evaporation of the solvent gave 0·357 g (81%) of the product.

Statistical analysis

Relaxations were 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 from the regression plot and a mean IC50 ± 95% confidence interval was calculated for each test compound.

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 assessed using Student's *t*-test for unpaired data. $P < 0.05$ were considered to represent significant differences.

Results

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

All the benzylisoquinolines produced dose-dependent relaxations in KCl-depolarized uterus; hence, dose-response (relaxation) curves were constructed by addition of cumulative doses of alkaloids (10^{-7}–10^{-4} M).

Fig. 2 shows these dose-response curves and Table 1 summarizes the maximal relaxation (E_{max}) and IC50 for each product tested. Papaveraldine shows relaxant activity only at

Table 1. Parameters of dose-response curves of relaxation induced by cumulative doses of isoquinoline derivatives on rat KCl-depolarized uterus.

	E_{max} (%)	IC50 (μM)	n
Papaverine	109·7±3·8	2·7±0·4	5
Papaverinol	102·2±5·8	6·3±2·8	6
Papaveraldine	16·4±4·1*	—	5
N-Methylpapaverine	53·3±8·8*	78·0±15·7*	6
Dihydropapaverine	93·7±4·5*	14·0±2·3*	6
Laudanosine	94·5±4·0*	17·2±3·3*	5
Glaucine	102·6±3·8	3·0±0·5	5

* $P \leq 0{\cdot}05$ vs papaverine.

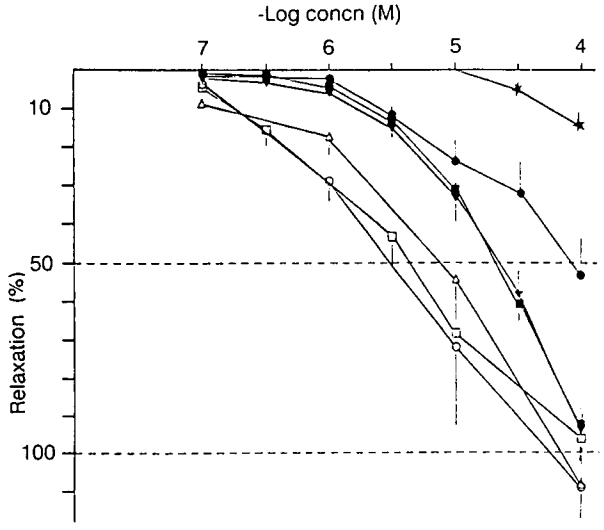


FIG. 2. Dose-response curves of relaxation to alkaloids in KCl-depolarized rat uterus. ○ Papaverine (n = 5), ● *N*-methylpapaverine (n = 6), △ papaverinol (n = 6), ★ papaveraldine (n = 5), ▲ dihydropapaverine (n = 6), ■ laudanosine (n = 5), □ glaucine (n = 5). Bars represent s.e.m. of n experiments.

the higher concentrations assayed, therefore, an IC50 value for this alkaloid could not be calculated.

The relaxant activity shown by papaverine, papaverinol and glaucine was significantly higher than the maximal relaxation achieved by the other compounds tested (*N*-methylpapaverine, dihydropapaverine and laudanosine; Table 1).

In the presence of propranolol (10^{-5} M), E_{max} and IC50 were not significantly modified for any of the products tested.

Modification of uterine response to oxytocin by papaverine and its derivatives

Addition of oxytocin 0·01 units mL^{-1} to the uterine horn incubated in Locke-Ringer solution induced rhythmic contractile response with stable frequency and amplitude. The addition of cumulative doses (10^{-7}–10^{-4} M) of alkaloids diminished both the frequency and amplitude of the contractions. To determine the inhibitory potency of each compound tested we analysed two parameters, the minimal effective dose that modified contractile responses to oxytocin, and the concentration of alkaloid that completely abolished these rhythmic contractions.

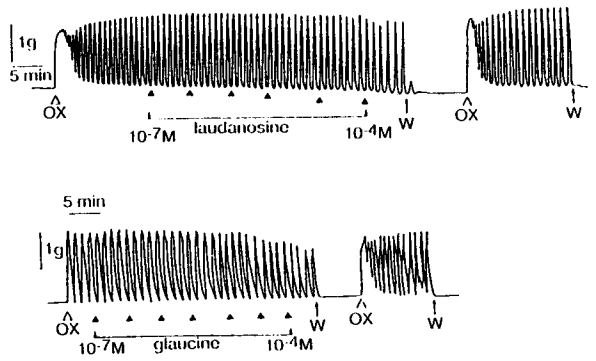


FIG. 3. Contractile responses induced by oxytocin (OX) 0·01 unit mL^{-1} in rat uterus. Addition of cumulative amounts of laudanosine or glaucine (10^{-7}–10^{-4} M) decreased the amplitude of contractile responses only with the highest concentration. After washing (W), contractions were again obtained by fresh addition of oxytocin.

As shown in Fig. 3, the minimal effective dose was 10^{-5} M (n = 6) for glaucine and higher for laudanosine (3×10^{-5} M; n = 6). The higher dose tested for each compound (10^{-4} M) produced only a slight decrease in the responses to oxytocin (n = 6). After washing, complete recovery of rhythmic contractions induced by oxytocin was observed.

The other alkaloids showed similar action. The minimal effective dose was 10^{-5} M for papaverine (D'Ocon et al 1989) and papaverinol (n = 5), 3×10^{-5} M for dihydropapaverine (n = 6) and *N*-methylpapaverine (n = 5) and 10^{-4} M for papaveraldine (n = 5). Only papaverine and papaverinol at the higher dose tested (10^{-4} M) completely abolished the contractile responses to oxytocin (n = 5).

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

Fig. 4 shows two examples of sustained uterine contractions induced by oxytocin (0·01 unit mL^{-1}) applied for a long

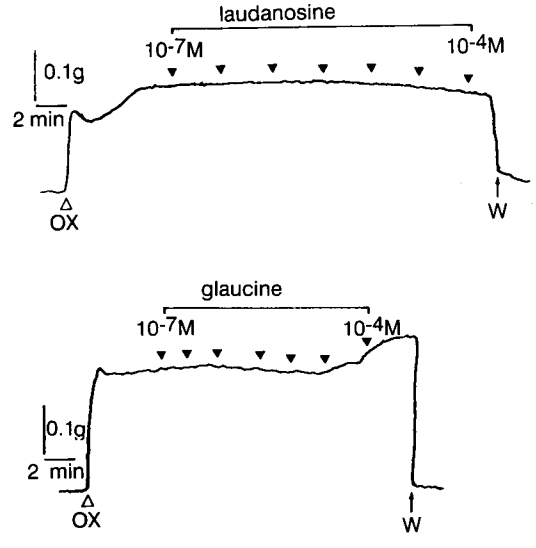


FIG. 4. Oxytocin (OX)-induced contraction of rat uterus in Ca^{2+}-free EDTA-containing solution. Addition of cumulative doses of laudanosine did not modify the contractile response to oxytocin. Glaucine caused an additional increase in tension with the highest concentration used.

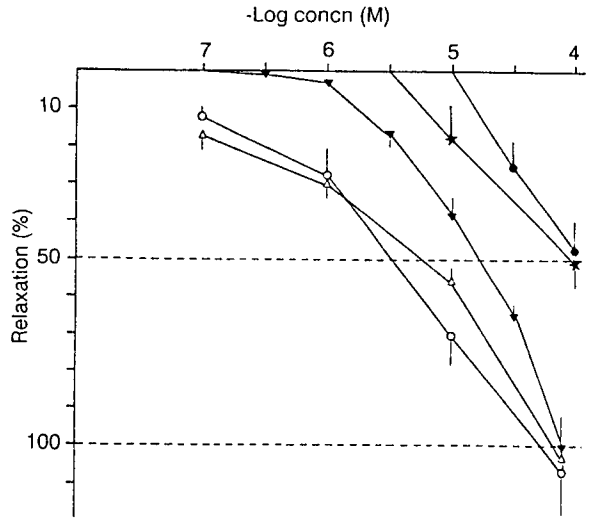


FIG. 5. Dose-response curves of relaxation to the alkaloids on the contractile response induced by oxytocin (0·01 unit mL^{-1}) in Ca^{2+}-free EDTA-containing solution. ○ Papaverine (n = 5), ● *N*-methylpapaverine (n = 5), △ papaverinol (n = 7), ★ papaveraldine (n = 5), ▼ dihydropapaverine (n = 5). Bars represent s.e.m. of n experiments.

period (> 15 min) after 20 min in Ca^{2+}-free medium; the amplitude of these contractions was 373·0 ± 38·8 mg (n = 13). When glaucine (10^{-7}–10^{-4} M) was added to the Ca^{2+}-free solution during the plateau of this contraction, there was an additional increase in tension (180·0 ± 39·6 mg; n = 5). In contrast, complete relaxation occurred when cumulative doses (10^{-7}–10^{-4} M) of papaverine (D'Ocon 1989), papaverinol or dihydropapaverine was added. The maximal relaxation and IC50 for each alkaloid are summarized in Table 2 and represented in Fig. 5.

Papaveraldine and *N*-methylpapaverine only partially relaxed the sustained contraction induced by oxytocin (Table 2). Addition of laudanosine in cumulative amounts did not modify the contractile plateau induced by oxytocin in Ca^{2+}-free medium (n = 6; Fig. 4).

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 562·3 ± 64·2 mg (n = 13). When alkaloids were added in cumulative amounts (10^{-7}–10^{-4} M) dose dependent relaxations were obtained by papaverine, papaverinol, papaveraldine, *N*-methylpapaverine and dihydropapaverine (Fig. 6, Table 3).

Table 2. Parameters of dose-response curves of relaxation induced by cumulative doses of isoquinoline derivatives on oxytocin contraction in Ca^{2+}-free medium.

	E_{max} (%)	IC50 (μM)	n
Papaverine	107·1 ± 10·2	3·2 ± 1·3	5
Papaverinol	103·8 ± 4·4	3·1 ± 0·6	7
Papaveraldine	51·5 ± 7·3*	148·6 ± 43·1*	5
N-Methylpapaverine	43·0 ± 11·2	—	5
Dihydropapaverine	105·0 ± 9·5	10·9 ± 0·9	5

*$P \leq 0{\cdot}05$ vs papaverine.

Table 3. Parameters of dose-response curves of relaxation induced by cumulative doses of isoquinoline derivatives on vanadate contraction in Ca^{2+}-free medium.

	E_{max} (%)	IC50 (μM)	n
Papaverine	125·7 ± 7·8	1·4 ± 0·3	4
Papaverinol	95·7 ± 2·4*	10·0 ± 4·0	5
Papaveraldine	33·7 ± 12·7*	—	6
N-Methylpapaverine	38·1 ± 1·8*	—	6
Dihydropapaverine	71·7 ± 4·5*	46·3 ± 1·8*	6

*$P \leq 0{\cdot}05$ vs papaverine.

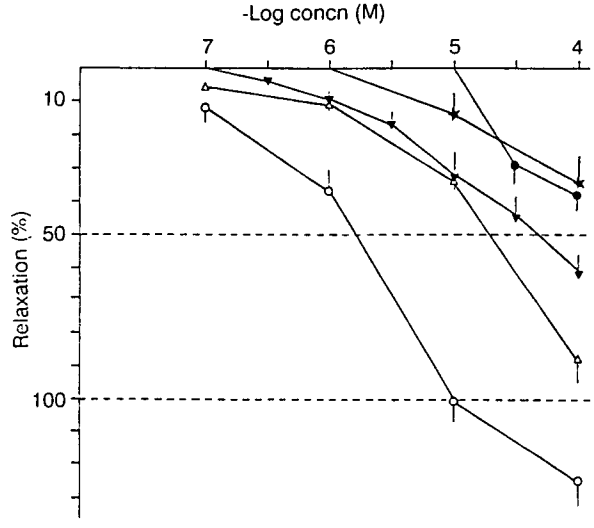


FIG. 6. Dose-response curves of relaxation of alkaloids on vanadate-induced contraction in Ca^{2+}-free EDTA-containing solution. ○ Papaverine (n = 4), ● *N*-methylpapaverine (n = 6), △ papaverinol (n = 5), ★ papaveraldine (n = 6), ▼ dihydropapaverine (n = 6). Bars represent s.e.m. of n experiments.

Addition of laudanosine did not modify the contractile response elicited by vanadate (n = 6; Fig. 7). The higher doses of glaucine (3×10^{-5} and 10^{-4} M) promoted an additional increase in tension when they were added during the vanadate-induced contractile plateau (117·8 ± 40·3 mg; n = 6; Fig. 7).

Discussion

Spasmogenic responses of the uterus to KCl can be entirely explained in terms of calcium influx from the extracellular fluid via potential-operated calcium channels (Amedée et al 1986; Edwards et al 1986). This mechanical response to KCl was completely inhibited by several Ca^{2+} entry blockers (Calixto & Loch 1985; Granger et al 1985, 1986; Ballejo et al 1986) or by incubation in Ca^{2+}-free medium (Granger et al 1986).

Conversely, the contraction induced by oxytocin in the myometrium is extremely resistant to Ca^{2+}-removal (Ashoori et al 1985; Villar et al 1985; Anselmi et al 1987; D'Ocon 1989) and in Ca^{2+}-containing solution, oxytocin at high concentrations induces a spasmogenic response that consists of two components, phasic and tonic, generated by different biochemical mechanisms (Edwards et al 1986). The

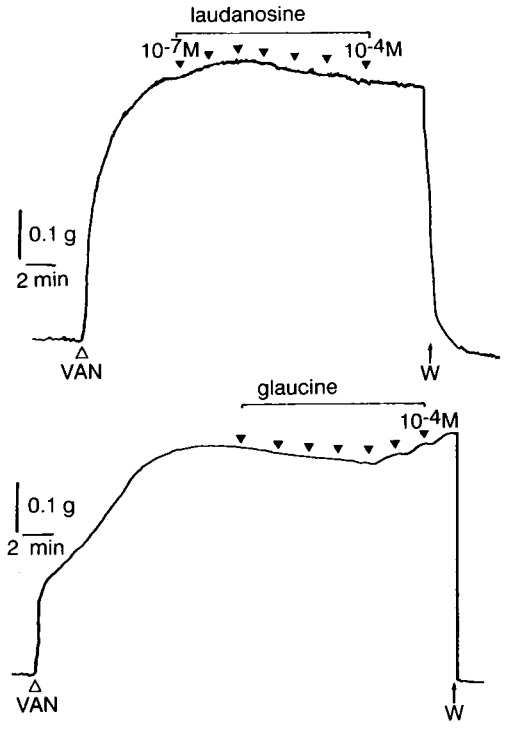


FIG. 7. Vanadate (VAN)-induced contraction of rat uterus in Ca^{2+}-free EDTA-containing solution. Addition of cumulative doses of laudanosine did not modify the contractile response to vanadate. Glaucine caused an additional increase in tension with the highest concentration used.

phasic component is related to Ca^{2+} influx, probably via POCs, and the tonic component involves the opening of receptor-operated channels or an increase in phosphatidyl inositol turnover, the subsequent release of Ca^{2+} from intracellular stores, decreased Ca^{2+} extrusion or decreased intracellular Ca^{2+} binding. The tonic component persists when oxytocin is added to the uterine preparation incubated in Ca^{2+}-free medium (Edwards et al 1986; Anselmi et al 1987; D'Ocon et al 1987; Savineau & Mironneau 1990).

Sustained contraction induced by vanadate in Ca^{2+}-free solution can also be related to release of Ca^{2+} from intracellular storage sites (D'Ocon 1989) and this action can be explained by the results of previous studies showing vanadate as a potent inhibitor of Ca-Mg ATPase (Mironneau et al 1984; Nechay 1984) and Na-K ATPase (Grover et al 1981).

On the basis of the above considerations, we studied the mechanism of the relaxant action of papaverine derivatives on uterine smooth muscle contraction induced by KCl, oxytocin or vanadate.

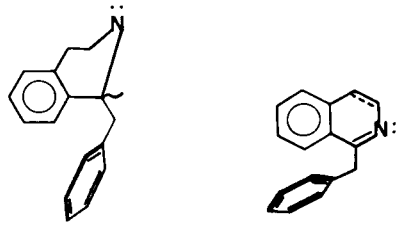
In previous studies (D'Ocon 1989; D'Ocon et al 1989) we have shown that papaverine relaxes the uterus previously contracted by either KCl and oxytocin in a medium containing Ca^{2+} or oxytocin and by vanadate in a Ca^{2+}-free medium. The finding that the IC50 of papaverine was similar in the experiments with and without Ca^{2+} in the medium would indicate that the inhibitory mechanism of papaverine is the same in both experimental conditions tested. These tests coincide with those suggested in previous works reporting that papaverine relaxes contractions produced by a number of stimulant substances acting at different sites. This action could be related to an increase in cAMP that leads to the accumulation of calcium in endoplasmic reticulum and induces phosphorylation of myosin light chain kinase, which causes smooth muscle relaxation (Cumiskey & Feigenson 1983; Calixto & Loch 1985).

Our results show that all the products tested inhibit both sustained K^+-induced contractions, and four of them inhibit rhythmic contractile responses to oxytocin in Ca^{2+}-containing solution in a concentration-related way. Glaucine and laudanosine only slightly modify the oxytocin-induced contractions. These potency differences indicate that the relaxant mechanism of glaucine and laudanosine may be related to Ca^{2+} influx possibly through POCs. The other compounds tested showed no differences in their capacity for inhibiting K^+- or oxytocin-induced contractile responses; in this respect they were similar to papaverine.

The present results indicate that glaucine and laudanosine act through a more specific mechanism related to nifedipine action (D'Ocón et al 1991). In order to confirm this hypothesis we assayed the relaxant effect of the alkaloids on contractile responses induced by oxytocin and vanadate in Ca^{2+}-free medium. The results obtained under our experimental conditions demonstrate that laudanosine and glaucine have no relaxant activity when they are added cumulatively to the uterine preparation previously contracted by both agonists in Ca^{2+}-free medium. Since the contractions obtained under these conditions are related only to release of Ca^{2+} from intracellular stores (see above), it is reasonable to postulate that glaucine and laudanosine do not have an intracellular site of action. Conversely, glaucine at the higher doses tested induced an increase in uterine tension, which suggests an additional release of stored calcium or an increase in the sensitivity of the contractile machinery.

Nifedipine, which relaxes contractile responses to KCl or oxytocin in uterus in Ca^{2+}-containing solution, fails to relax sustained contractions induced by oxytocin or vanadate in Ca^{2+}-free medium (D'Ocon et al 1991). It is clear from the present study that the behaviour of glaucine and laudanosine is similar to nifedipine; hence, we believe that both alkaloids inhibit Ca^{2+}-influx via POCs, as does nifedipine, as it can be assumed that contractions evoked by KCl in smooth muscle are directly related to the influx of Ca^{2+} into the cell, specifically through L-channels (Amedée et al 1986; Martin et al 1989). Similar results were obtained in the benzylisoquinoline or bisbenzylisoquinoline series with compounds such as cularines, isocrasifoline (D'Ocon et al 1991), aporfines (Cortes et al 1990) or antioquine (D'Ocon et al 1989). These alkaloids also have a more specific activity closely related to the inhibition of calcium entry via POCs. The structural characteristic common to these alkaloids with specific activity is the presence of a tetrahydroisoquinoline ring (Fig. 1).

However, the results of previous studies (D'Ocon et al 1989; D'Ocon 1989) and those presented here suggest that unsaturated benzylisoquinolines (papaverine, papaverinol, papaveraldine, *N*-methylpapaverine and dihydropapaverine) relax uterine smooth muscle through a mechanism closely related to intracellular action on Ca^{2+} levels. The fact that the IC50 of all the compounds tested was similar with and without Ca^{2+} in the medium would indicate that their



A B

FIG. 8. A. Benzyltetrahydroisoquinoline. B. Benzylisoquinoline.

relaxant action does not depend exclusively on a blockade of calcium influx.

On the basis of the characteristics of the relaxant action we can divide the alkaloids tested into those containing an unsaturated heterocyclic ring and those with a tetrahydroisoquinoline ring, the latter group exhibiting a more specific activity similar to that of nifedipine.

Instead of an sp^2-like nitrogen these compounds have an sp^3-like hybridized nitrogen atom that allows the existence of different types of interaction between the nitrogen electron pair and the aromatic rings or the electrophilic receptor sites. This different interaction might be attributed to the geometry as well as to differences in the basicity of the heteroatom (Fig. 8).

The similar activities of (−)-glaucine and (±)-laudanosine strongly suggests that restriction of the free rotation of the benzylic group does not play a key role in activity. The rigid (−)-glaucine was found to be more potent than (±)-laudanosine and this difference could be related to the absolute configuration *S* at the chiral carbon atom in (−)-glaucine or to the fixed position occupied by the benzyl group relative to nitrogen.

The selectivity observed in the case of laudanosine and glaucine implies an action on the Ca^{2+} influx from the extracellular medium without changes in the intracellular distribution of this ion. Present results agree well with previous reports showing that the hydrogenation of the heterocyclic ring of benzylisoquinoline gives a 10- to 20-fold reduction in potency as a cyclic nucleotide phosphodiesterase inhibitor (Van Inwegen et al 1979). This observation could explain the lack of an intracellular action of the two tetrahydroisoquinolines tested. However, the potencies of glaucine and laudanosine as inhibitors of KCl-induced contraction were found to be similar to that of papaverine.

In the group of benzylisoquinolines containing an unsaturated heterocyclic ring we also tested the influence of the degree of oxidation of the benzylic carbon. The introduction of a hydroxy group at this position does not modify the activity. By contrast, the oxidation to ketone gives rise to a reduction of the activity as important as that due to quaternization of the nitrogen atom. The quaternization enhances the polarity of the molecule and inhibits the basic character of the nitrogen atom. The oxidation to ketone of the benzylic carbon modifies the shape of the molecule, enhances the polarity and diminishes the basicity of the quinoline ring.

We conclude that unsaturated benzylisoquinolines exhibit a common feature in relaxing uterine smooth muscle by altering the distribution of intracellular Ca^{2+}, increasing Ca^{2+}-efflux or decreasing the sensitivity of the contractile system.

Acknowledgement

This work was supported by Grant No. PB86-0462 from the Spanish Commision Interministerial de Ciencia y Tecnología.

References

Anselmi, E., D'Ocon, M. P., Villar, A. (1987) A comparison of uterine contraction induced by PGE_1 and oxytocin in Ca-free solution. Prostaglandins 34: 351–358

Amedée, T., Mironneau, C., Mironneau, J. (1986) Isolation and contractile responses of single pregnant rat myometrial cells in short-term primary culture and the effects of pharmacological and electrical stimuli. Br. J. Pharmacol. 88: 873–880

Ashoori, E., Takai, A., Tomita, T. (1985) The response of non-pregnant myometrium to oxytocin in Ca-free solution. Ibid. 84: 175–183

Ballejo, G., Calixto, J. B., Medeiros, Y. S. (1986) "In vitro" effects of calcium entry blockers, chlorpromazine and fenoterol upon human pregnant myometrium contractility. Ibid. 89: 515–523

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

Calixto, J. B., Loch, S. (1985) Ketamine-inhibition of calcium-induced contractions in depolarized rat uterus: a comparison with other calcium antagonists. Br. J. Pharmacol. 85: 189–195

Cortes, D., Torrero, M. Y., D'Ocon, M. P., Candenas, M. L., Cavé, A., Hadi, A. H. A. (1990) Norstephalagine et atherospermidine, deux aporphines *d'Artabotrys maingayi* relaxantes du muscle lisse. J. Nat. Prod. 53: 503–508

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

D'Ocon, M. P. (1989) Different effects of papaverine, isoprenaline and caffeine on rat uterine contractility in Ca^{2+}-free medium. Ibid. 302: 268–279

D'Ocon, M. P., Anselmi, E., Villar, A. (1987) Effects of magnesium chloride on the contractile response of uterus to several agonists in Ca^{2+}-free solution. J. Pharm. Pharmacol. 39: 444–448

D'Ocon, M. P., Candenas, M. L., Anselmi, E., Zafra-Polo, M. C., Cortes, D. (1989) Antioquine: a new bisbenzylisoquinoline alkaloid with calcium antagonist activity. Arch. Int. Pharmacodyn. 297: 205–216

D'Ocon, M. P., Blasco, R., Candenas, M. L., Ivorra, M. D., Lopez, S., Villaverde, C., Castedo, L., Cortes, D. (1991) Relaxant effects of cularines and isocrasifoline on smooth muscle contractions: interactions with calcium entry through POCs. Eur. J. Pharmacol. 196: 183–187

Edwards, D., Good, D., Granger, S., Hollingsworth, M., Robson, A., Small, R. C., Weston, A. H. (1986) The spasmogenic action of oxytocin in the rat uterus—comparison with other agonists. Br. J. Pharmacol. 88: 899–908

Godfraind, T., Miller, R., Wibo, M. (1986) Calcium antagonism and calcium entry blockade. Pharm. Rev. 38: 321–415

Granger, S. E., Hollingsworth, M., Weston, A. H. (1985) A comparison of several calcium antagonists on uterine vascular and cardiac muscles from the rat. Br. J. Pharmacol. 85: 255–262

Granger, S. E., Hollingsworth, M., Weston, A. H. (1986) Effects of calcium entry blockers on tension development and calcium influx in rat uterus. Ibid. 87: 147–156

Grover, A. K., Kwan, C. N., Daniel, E. E. (1981) Na-Ca exchange in rat myometrium membrane vesicles highly enriched in plasma membranes. Am. J. Physiol. 240: C175–178

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

Martin, C., Dacquet, C., Mironneau, C., Mironneau, J. (1989) Caffeine-induced inhibition of calcium channel current in cultured

smooth muscle from pregnant rat myometrium. Br. J. Pharmacol. 98: 493–498

Mironneau, C., Mironneau, J., Savineau, J. P. (1984) Maintained contractions of rat uterine smooth muscle incubated in a Ca-free solution. Ibid. 82: 735–743

Nechay, B. R. (1984) Mechanisms of action of vanadium. Annu. Rev. Pharmacol. 24: 501–524

Savineau, J. P., Mironneau, J. (1990) Caffeine acting on pregnant rat myometrium: analysis of its relaxant action and its failure to release Ca from intracellular stores. Br. J. Pharmacol. 99: 261–266

Triggle, D. J., Langs, D. A., Janis, R. A. (1989) Ca-channels ligands: structure-function relationships of the 1,4-dihydropyridines. Med. Res. Rev. 9: 123–180

Van Inwegen, R. G., Salaman, P., St. Georgiev, V., Weinryb, I. (1979) Dihydro- and tetrahydroisoquinolines as inhibitors of cyclic nucleotide phosphodiesterases from dog heart. Biochem. Pharmacol. 28: 1307–1312

Villar, A., D'Ocon, M. P., Anselmi, E. (1985) Calcium requirement of uterine contraction induced by PGE_1: importance of intracellular calcium stores. Prostaglandins 30: 491–496