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Different Mechanism of Relaxation Induced by Aporphine Alkaloids in Rat Uterus

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Abstract—We have examined the uterine relaxant action of three aporphine molecules (*S*-glaucine, *S*-boldine and *R*-apomorphine) in two experimental conditions, with and without calcium in the bathing solution, and compared these effects with those obtained with the calcium antagonists verapamil and diltiazem. The present study shows that the alkaloids relax the uterine muscle but with different mechanisms of action. In Ca^{2+}-containing solution all three alkaloids relaxed the uterus previously contracted by KCl or acetylcholine, but in Ca^{2+}-free medium only *R*-apomorphine was able to relax oxytocin-induced contraction. The calcium antagonists, verapamil and diltiazem, relaxed KCl- or acetylcholine-induced contraction in Ca^{2+}-containing solution, whereas they only relaxed oxytocin-induced contraction in Ca^{2+}-free medium at much higher doses. These results suggest that glaucine and boldine behave as specific calcium entry blockers without affecting the contractile machinery or intracellular Ca^{2+} levels as apomorphine does. The absolute configuration (*S*-glaucine and *S*-boldine vs *R*-apomorphine) may account for this different action.

New types of calcium antagonists, unlike the well-known phenylalkylamines, dihydropyridines and benzothiazepine derivatives, have recently been identified (Rampe & Triggle 1990). It has been demonstrated that among these compounds there is a bisbenzyltetrahydroisoquinoline alkaloid, tetrandrine, which interacts at the benzothiazepine binding site of the L-channel (King et al 1988). Another bisbenzyltetrahydroisoquinoline, antioquine (D'Ocon et al 1989; Ivorra et al 1992a), and other benzyltetrahydroisoquinoline molecules such as cularines, isocrasifoline (D'Ocon et al 1991) and some aporphines (Anselmi et al 1992) have been described in rat uterus as specific relaxant agents with a mechanism of action closely related to inhibition of Ca^{2+} influx via voltage-operated Ca^{2+}-channels. In spite of structural similarity, the mechanism of action exhibited by these molecules is different from the non-specific relaxant agent papaverine.

Among these compounds we have demonstrated in previous work using radiologand binding techniques that the aporphine alkaloids, glaucine (Ivorra et al 1992b), boldine and apomorphine (Ivorra et al 1993) acted at the benzothiazepine site in the calcium channel as well as at the α_1-adrenergic receptor in rat cortical membranes. These compounds exhibited both calcium antagonist and α_1-adrenergic properties in rat aorta. Glaucine and boldine acted as α_1-adrenergic antagonists whereas apomorphine seemed to act as a partial agonist (Ivorra et al 1992b, 1993).

In the present work we have studied the relaxant effect of these three aporphine molecules (glaucine, boldine and apomorphine) in another smooth muscle: the rat uterus. Glaucine and boldine are aporphine molecules with a chiral *S* centre and only differ in the degree of methylation at the hydroxy groups. In contrast, apomorphine exhibits an *R* chiral centre, has two hydroxy groups in the benzyl ring and no substituents in the isoquinoline ring (Fig. 1). For comparison the effect of the two calcium antagonists, diltiazem and verapamil were evaluated in the same experimental conditions as the alkaloids.

We have assayed the uterine relaxant effect of these alkaloids on the maintained contraction induced by different agonists in two experimental conditions, with and without calcium in the extracellular medium. In calcium-containing solution, KCl and acetylcholine were used as spasmogens. K^+-depolarization is known to induce smooth muscle contractions by promoting the influx of extracellular calcium through the voltage-operated channel (Bolton 1979; Ballejo et al 1986; Edwards et al 1986; Granger et al 1986; Godfraind

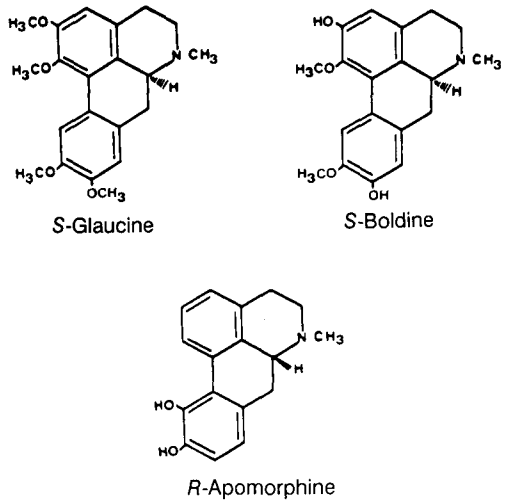

FIG. 1. Molecular formulae of *S*-glaucine, *S*-boldine and *R*-apomorphine.

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et al 1986). In contrast, contractions induced by acetylcholine are due partially to calcium release by intracellular stores and partially to influx of extracellular Ca^{2+} (Bolton 1979; Godfraind et al 1986). The channel-opening by muscarinic receptor activation seems to be voltage-sensitive (Bolton et al 1990). In Ca^{2+}-free medium, oxytocin was used as a spasmogen since it can induce a sustained contraction (Edwards et al 1986; Anselmi et al 1987; D'Ocon et al 1987a; Savineau & Mironneau 1990), whereas KCl did not induce any contraction (Ballejo et al 1986; Villar et al 1986) and acetylcholine induced only a single transient contraction of uterus (D'Ocon et al 1987b).

Materials and Methods

Preparation of uterine horns

Female Wistar rats, 150–200 g, given oestradiol benzoate (5 mg kg^{-1}) were killed 24 h later by a blow on the head and exsanguinated. A segment from each uterine horn was mounted in a 10 mL organ bath with physiological solution bubbled with 95% O_2–5% CO_2 at 31°C to avoid the spontaneous activity of the uterus as previously reported (Villar et al 1986; Anselmi et al 1987, 1992; D'Ocon et al 1987a,b, 1989).

Effects of agents on tonic contractions induced by KCl or acetylcholine

The ability of the compounds to relax the maintained tonic phase of contractions induced by KCl or acetylcholine was examined. The organ was immersed in Jalon-Ringer solution and equilibrated for 20 min under a resting tension of 1 g. After the equilibration period, the preparation was contracted by changing the solution in the bath to a depolarizing solution (56 mM KCl) or by the addition of acetylcholine to the bath to a final concentration of 10^{-4} M.

When maximal maintained tonic contractions to these spasmogens were obtained, the alkaloids (glaucine, boldine and apomorphine) or the calcium channel antagonists (verapamil and diltiazem) were added cumulatively to the tissue bath, and concentration-related relaxations were observed. 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 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 from the linear regression plot of all points between 20–80% of the maximal response.

Effects of agents on oxytocin-induced Ca^{2+}-free contraction

Another set of experiments was carried out without extracellular calcium in order to study a possible action of these compounds at the intracellular level. A uterine horn was equilibrated for 1 h in Ringer-Locke 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 a further 20 to 30 min. Sustained contractile response to oxytocin (0·01 units mL^{-1}) was 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

Two different solutions were used when the experiments were carried out in the presence or absence of Ca^{2+}. When the experiments were made in Ca^{2+}-containing medium, Jalon-Ringer containing a lower Ca^{2+} concentration was used to avoid the spontaneous activity shown by the uterus when it is exposed to physiological Ca^{2+} concentrations (2·16 mM: Ringer-Locke solution). In the experiments without Ca^{2+} we used the solution and the protocol reported previously by Sakai et al (1981, 1982).

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

Drugs and chemicals

Verapamil, diltiazem, glaucine, boldine, apomorphine and oxytocin were purchased from Sigma Chemical Co. All aqueous solutions (pH 7) were prepared daily. All chemicals used were of analytical grade.

Statistical analysis

The results are expressed as the mean ± s.e.m. of 5 or more preparations (n) obtained from different animals. The statistical significance of differences between means was assessed using Student's t-test and $P<0·05$ was considered significant.

Results

Effect of alkaloids and calcium channel blockers on the KCl-induced contractions

KCl (56 mM) caused a rapid phasic contraction, followed by a slight relaxation and a prolonged tonic contraction. Addition of cumulative amounts of glaucine, boldine and apomorphine (10^{-7}–3×10^{-4} M) and of calcium antagonists, verapamil and diltiazem (10^{-8}–10^{-4} M) evoked dose-related relaxations in the maintained contractions induced by KCl. The concentration-response curves are shown in Fig. 2 and the parameters of these curves are summarized in Table 1. All the agents tested produced a 100% relaxation of the contractions induced by KCl and the rank order of potency was verapamil > diltiazem > glaucine > apomorphine > boldine.

After addition of cumulative amounts of alkaloids (glaucine, boldine and apomorphine) and subsequent washing of the uterus, a second addition of KCl produced a contraction with the same morphology as the first but with a significantly smaller magnitude (41·6 ± 4·8% after glaucine, 52·9 ± 5·7% after boldine, and 32·1 ± 5·6% after apomorphine). In contrast, after treatment with the calcium antagonists no recovery of contraction to KCl was observed.

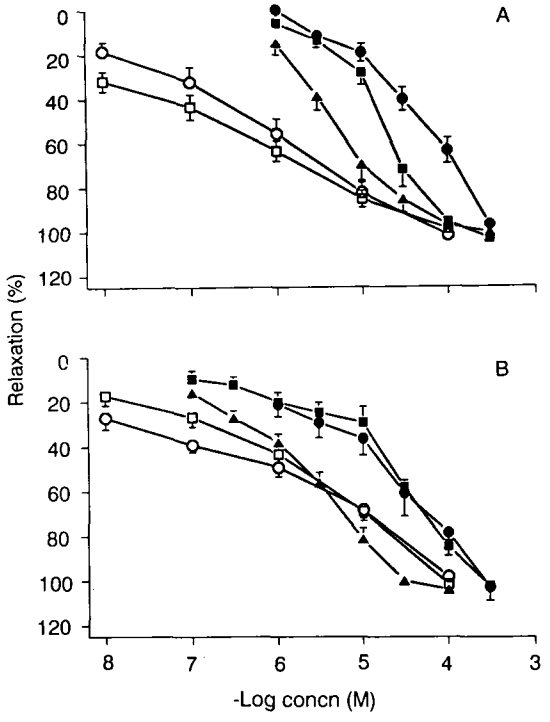

FIG. 2. Dose-response relaxation curves obtained after the addition of different agents in uterus previously contracted with 56 mM KCl (A) or 10^{-4} M acetylcholine (B). □ Verapamil; ○ diltiazem; ▲ glaucine; ● boldine; ■ apomorphine. Each point is the mean derived from n experiments with s.e.m. shown by vertical bars.

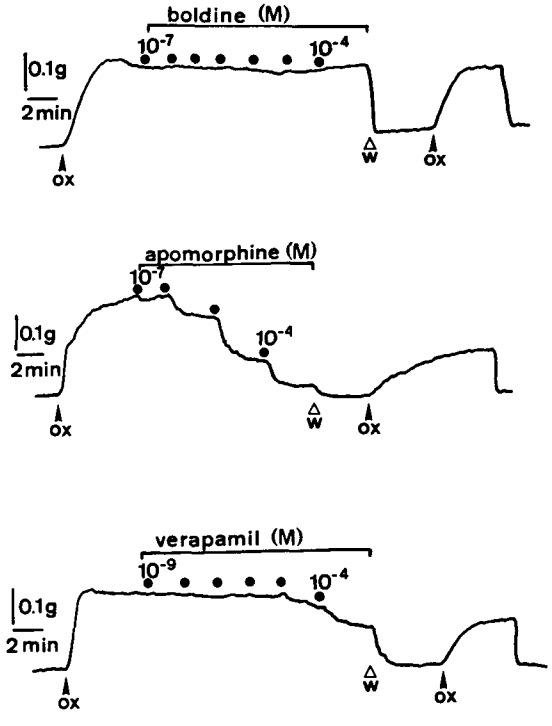

FIG. 3. Contractile response to oxytocin (OX) 0·01 units mL^{-1} in uterus incubated in Ca^{2+}-free EDTA-containing solution was relaxed by the addition of cumulative doses of apomorphine. The addition of cumulative doses of boldine did not modify the contractile response to oxytocin. Verapamil relaxed the oxytocin-induced contraction only with the highest dose tested. W = washout.

Effect of alkaloids and calcium channel blockers on the acetylcholine-induced contractions

Acetylcholine (10^{-4} M) induced an initial phasic contraction followed by a plateau with rhythmic contractions. Addition of cumulative amounts of glaucine, boldine and apomorphine (10^{-7}–3×10^{-4} M) and of calcium antagonists, verapamil and diltiazem (10^{-8}–10^{-4} M) evoked dose-related relaxations in the maintained contractions induced by acetylcholine. The rhythmic contractions induced by acetylcholine disappeared after the addition of different agents. The concentration-response curves are shown in Fig. 2 and the parameters of these curves are summarized in Table 1. All the agents tested produced a 100% relaxation of the contractions induced by acetylcholine and the IC50 values at which the different compounds relaxed acetylcholine-induced tone were similar to those needed to relax KCl-induced tone. Irrespective of the agonist employed to contract the uterus, glaucine was the most potent of the alkaloids tested. Apomorphine was 3–4 times less potent than glaucine, while boldine was consistently the least potent agent tested.

After addition of cumulative amounts of alkaloids in the maintained contractions induced by acetylcholine and subsequent washing of the uterus, a new addition of acetylcholine induced a contractile response significantly smaller than the first (58·2 ± 5·4% after glaucine, 89·1 ± 4·6% after boldine, 74·5 ± 5·8% after apomorphine) but with a similar morphology. However, after treatment with the calcium

Table 1. Parameters of dose-response curves for the relaxation induced by cumulative doses of different agents tested in rat uterus previously contracted with 56 mM KCl or 10^{-4} M acetylcholine.

	KCl			Acetylcholine		
	E_{max} (%)	IC50 (μM)	n	E_{max} (%)	IC50 (μM)	n
Verapamil	99·0 ± 0·7	0·49 ± 0·18	12	101·5 ± 0·7	1·21 ± 0·28*	10
Diltiazem	101·9 ± 1·8	1·01 ± 0·24	10	98·0 ± 2·1	0·86 ± 0·20	9
Glaucine	100·5 ± 2·6	4·00 ± 0·97	8	103·9 ± 1·4	1·78 ± 0·40	8
Boldine	97·1 ± 1·9	32·5 ± 9·4†	9	102·7 ± 6·0	24·9 ± 10·1†	7
Apomorphine	103·4 ± 1·3	14·1 ± 2·7†	11	102·1 ± 0·8	14·5 ± 3·3†	8

Data represents the mean ± s.e.m. n = number of experiments. *Significant differences ($P < 0·05$) from the corresponding values on K^+-induced contraction and †significant differences from glaucine.

antagonists, verapamil and diltiazem, a second addition of acetylcholine induced a contraction that was different from the first in both morphology and magnitude: only a phasic component of the response, which was significantly smaller than that obtained in the first application (37·8 ± 9·4% after verapamil, 39·6 ± 8·7% after diltiazem) could be observed; moreover in this second response to acetylcholine, the typical rhythmic contractions induced by this agonist did not appear.

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

Fig. 3 shows that the sustained contraction caused by oxytocin in Ca^{2+}-free EDTA-containing solution was completely abolished by the addition of cumulative concentrations of apomorphine (10^{-7}–10^{-4} M) with an IC50 value of 5·37 ± 1·75 μM and a maximal relaxation of 82·0 ± 6·2% (n = 4). However, the addition of similar cumulative concentrations of glaucine (Anselmi et al 1992) or boldine (10^{-7}–10^{-4} M) did not modify the contraction plateau induced by oxytocin (n = 3). The calcium antagonists, diltiazem (10^{-11}–10^{-3} M) (Ivorra et al 1992a) and verapamil (10^{-9}–10^{-4} M) relaxed the oxytocin-induced contraction only in a Ca^{2+}-free medium with the highest dose tested (Fig. 3).

Discussion

In an attempt to elucidate the relationship between the structure and activity of benzylisoquinoline alkaloids, we demonstrated in earlier studies that the compounds with a tetrahydroisoquinoline ring exhibit a more specific uterine relaxant activity (closely related to inhibition of Ca^{2+} influx via voltage-operated Ca^{2+}-channels without any intracellular effect) than that shown by the compounds, such as papaverine, which contain an unsaturated heterocyclic ring (D'Ocon et al 1991; Anselmi et al 1992). The structural features of these specific relaxant compounds that define their geometry are the presence of an sp^3-like hybridized nitrogen atom, a chiral centre and a partially flexible tetrahydroisoquinoline ring. Moreover, it has also been demonstrated that in the series of bisbenzylisoquinoline alkaloids, not only the presence of a tetrahydroisoquinoline ring but also the absolute configuration of the chiral carbon may determine the specificity of their relaxant uterine action (D'Ocon et al 1992; Ivorra et al 1992a). However, in rat aorta we have previously demonstrated that three aporphine alkaloids with a tetrahydroisoquinoline ring but with a different absolute configuration at the chiral carbon (*S*-glaucine, *S*-boldine and *R*-apomorphine) acted as calcium antagonists and at the α_1-adrenoceptor level. Glaucine and boldine acted as α_1-adrenergic antagonists whereas apomorphine seems to behave as a partial agonist (Ivorra et al 1992b, 1993). In the present work we have examined the mechanism of action in rat uterus of these three alkaloids.

The three alkaloids tested were equipotent in inhibiting contractions of uterine muscle induced by KCl or acetylcholine. In both experimental conditions the rank order of potency was glaucine > apomorphine > boldine. Since glaucine and boldine only differ in the degree of methylation, the present results corroborate those of previous studies on other benzylisoquinoline alkaloids (D'Ocon et al 1991; Ivorra et al 1992a) in that an increase in the degree of methylation of the hydroxy groups (glaucine vs boldine) enhances the relaxant activity.

In the same experimental conditions similar results were obtained with the calcium antagonists, verapamil and diltiazem. These compounds also inhibit in a concentration-related way both KCl and acetylcholine-induced contractions of rat uterine smooth muscle, although verapamil was slightly less effective in blocking the contraction induced by acetylcholine. The order of potency of verapamil and diltiazem against K^+-induced contractions in rat uterus was compatible with the potency observed in some other smooth muscle preparations (Godfraind et al 1986).

To assess the possible intracellular action of the alkaloids, we have also tested the effect of the different compounds in Ca^{2+}-free medium. In these experimental conditions the behaviour of the three alkaloids was not the same. Glaucine (Anselmi et al 1992) and boldine did not act intracellularly, while apomorphine was able to relax the oxytocin-induced contraction in Ca^{2+}-free solution that is related to the liberation of Ca^{2+} from the intracellular stores (Anselmi et al 1987; Anwer & Sanborn 1989; Carsten & Miller 1987; D'Ocon 1989). It should be noted that the IC50 value of the spasmolytic action of apomorphine in intact muscle bathed in Ca^{2+}-containing solution is similar to that obtained in the uterus incubated in Ca^{2+}-free medium, which suggests that when this compound acts intracellularly, it fully relaxes tissues. Similar results were obtained in rat uterus when papaverine and related compounds with an unsaturated heterocyclic ring were tested (D'Ocon et al 1991; Anselmi et al 1992).

In the same experimental conditions, the calcium antagonists verapamil and diltiazem only relax the oxytocin-induced contraction at high concentrations ($> 10^{-5}$ M). An intracellular action of diltiazem at high doses has been reported by Hirano et al (1990) in coronary arteries.

Taken together, these results provide functional evidence that in rat uterus, glaucine and boldine behave as specific calcium entry blockers and do not interfere with contractile machinery, whereas apomorphine acts at the intracellular level. We recently demonstrated that the three alkaloids act at the benzothiazepine receptor site in the calcium channel, using radioligand binding experiments in rat cerebral cortex (Ivorra et al 1992b, 1993) and this action may be responsible for the selective relaxant effect showed for glaucine and boldine in the present work. However, apomorphine in rat uterus should have an additional effect at the intracellular level. The absolute configuration at the chiral carbon atom (*S*-glaucine and *S*-boldine vs *R*-apomorphine) may account for the different mechanism of relaxant action of these alkaloids, as previously reported for other bisbenzylisoquinolines (D'Ocon et al 1992; Ivorra et al 1992a) but we cannot exclude the possibility that the different substituents of glaucine and boldine vs apomorphine may also be responsible for this different action.

However, as can be seen in Fig. 4, when the molecular conformations of these alkaloids were determined by the MMX molecular-mechanics calculation program, the presence or absence of substituents did not significantly modify the structural conformation either in the *S*- or *R*-series whereas a difference in the molecules was determined by the

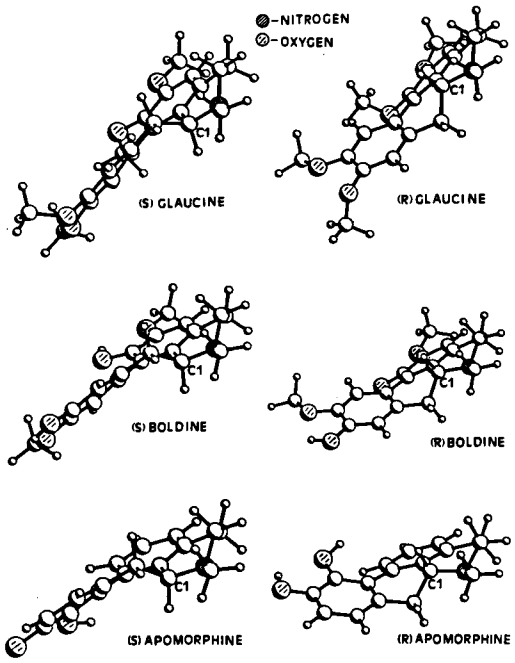

FIG. 4. Perspective views of the molecular conformation of the alkaloids determined by the MMX program from Serena Software Ltd, Bloomington, IN; MMX molecular-mechanics programs were established from the MM2 and MMP1 programs (Allinger QCPE 395 and QCPE 318) by K. E. Gilbert and J. J. Gajewski.

S or *R* configuration at the chiral centre. This suggests that the different action shown by glaucine and boldine vs apomorphine may be attributed to the conformational structure determined by the chiral carbon rather than to a difference in the substituents.

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References

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

Anselmi, E., Fayos, G., Blasco, R., Candenas, L., Cortes, D., D'Ocon, P. (1992) Selective inhibition of calcium entry induced by benzylisoquinolines in rat smooth muscle. J. Pharm. Pharmacol. 44: 337–343

Anwer, K., Sanborn, B. M. (1989) Changes in intracellular free calcium in isolated myometrial cells: role of extracellular and intracellular calcium and possible involvement of guanine nucleotide sensitive proteins. Endocrinology 124: 17–23

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

Bolton, T. B. (1979) Mechanism of action of transmitters and other substances on smooth muscle. Physiol. Rev. 85: 606–718

Bolton, T. B., Beech, D. J., Komori, S., Prestwich, S. A. (1990) Voltage- and receptor gated channels. In: Sperelakis, N., Wood, J. D. (eds) Frontiers in Smooth Muscle Research. Alan R. Liss Inc New York, pp 229–243

Carsten, M., Miller, J. D. (1987) A new look at uterine muscle contraction. Am. J. Obstet. Gynecol. 157: 1303–1315

D'Ocon, M. P. (1989) Different effects of papaverine, isoprenaline and caffeine on rat uterine contractility in Ca-free medium. Arch. Int. Pharmacodyn. Ther. 302: 268–279

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

D'Ocon, M. P., Anselmi, E., Villar, A. (1987b) Role of stored Ca in contractions of rat uterus induced by acetylcholine: effects of preincubation with Mg-free solution. Arch. Int. Pharmacodyn. Ther. 286: 162–170

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. Ther. 297: 205–216

D'Ocon, M.P., Blasco, R., Candenas, L., Ivorra, M. D., López, S., Villaverde, C., Castedo, L., Cortes, D. (1991) Inhibition of calcium entry induced by cularines and isocrasifoline in uterine smooth muscle. Eur. J. Pharmacol. 196: 183–187

D'Ocon, P., Blazquez, 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

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. (1986) Effects of calcium entry blockers on tension development and calcium influx in rat uterus. Br. J. Pharmacol. 87: 147–156

Hirano, K., Kanaide, H., Shimako, A., Nakamura, M. (1990) Effects of diltiazem on calcium concentrations in the cytosol and on force of contractions in porcine coronary arterial strips. Br. J. Pharmacol. 101: 272–280

Ivorra, M. D., Cercos, A., Zafra-Polo, M. C., Pérez-Prieto, J., Saez, J., Cortes, D., D'Ocon, M. P. (1992a) Selective chiral inhibition of calcium entry promoted by bisbencyltetrahydroisoquinoleines in rat uterus. Eur. J. Pharmacol. 219: 303–309

Ivorra, M. D., Lugnier, C., Schott, C., Catret, M., Noguera, M. A., Anselmi, E., D'Ocon, M. P. (1992b) Multiple actions of glaucine on cyclic nucleotide phosphodiesterases, α_1-adrenoceptor and benzothiazepine binding site at the calcium channel. Br. J. Pharmacol. 106: 387–394

Ivorra, M. D., Chuliá, S., Lugnier, C., D'Ocon, M. P. (1993) Selective action showed by two apophines at α_1-adrenoceptors and in the potential-operated calcium channel. Eur. J. Pharmacol. 231: 165–174

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 slowly inactivating calcium channels. J. Biol. Chem. 263: 2238–2244

Rampe, D., Triggle, D. J. (1990) New ligands for L-type Ca^{2+} channels. Trends Pharmacol. Sci. 11: 112–115

Sakai, K., Higuchi, K., Yamaguchi, T., Uchida, M. (1981) Oxytocin-induced Ca^{2+} free contraction of rat uterine smooth muscle: effects of divalent cations and drugs. Arch. Int. Pharmacodyn. 250: 40–54

Sakai, K., Higuchi, K., Yamaguchi, T., Uchida, M. (1982) Oxytocin-induced Ca^{2+} free contraction of rat uterine smooth muscle: effects of preincubation with EGTA and drugs. Gen. Pharmacol. 13: 393–399

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

Villar, A., D'Ocon, P., Anselmi, E. (1986) Role of intracellular calcium stores in the contractile response of uterus to several agonists. J. Pharmacol. 17: 541–546