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Uterine inhibitory effect of reticuline

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Abstract—Reticuline, the most abundant benzylisoquinoleic alkaloid of *Laurobasidium lauri*, exerts a uterine inhibitory effect mainly related to a decrease in the concentration of cytosolic calcium available for contraction.

The fungal species *Laurobasidium lauri* (Geyler) Jülich, is used in folk medicine on the Island of Madeira for the treatment of circulatory disturbances, skin rashes, nervous alterations, rheumatic and lumbar pains and specially in the treatment of uterine disturbances.

Different extracts of this fungus have an important uterine inhibitory effect (J. A. Sagredo, unpublished). According to results obtained previously, the chemical principles responsible for this action seem to be alkaloids of which reticuline is the major component in this fungus.

This alkaloid is a precursor in the biosynthesis of morphine and has a similar structure to papaverine, a well-known inhibitor of smooth muscle.

Materials and methods

Uterine preparations. Uterine strips of 1·5–2·0 cm length were obtained from Wistar rats, 150–200 g. The rats had been treated with oestradiol benzoate (1 mg kg^{-1} s.c.) 24 h before death. The strips were placed in a 10 mL capacity bath with a suitable nutrient solution at 30°C gassed with 5% CO_2 in O_2. Initial tension was 0·5 g, the isometric recording being measured by a Letica transducer coupled to a Letica UNIGRAPH LI 1000–100 ISO recorder. The nutrient solution, the time of stabilization and the working conditions were varied according to the type of assay to be performed.

Assays in uteri depolarized by potassium. The preparations were kept in De Jalon solution (De Jalon et al 1945) until a stable baseline was obtained, after which the nutrient solution was substituted by Ringer hyperpotassic solution which depolarized the membrane and contracted the muscle until a stable plateau was attained (Villar et al 1985). Once the depolarization plateau had been reached, reticuline was added at increasing cumulative doses. The results were compared with verapamil, a well-known inhibitor of voltage-dependent calcium channels (Goleenhofen & Lammel 1972; Mikkelsen et al 1978), and papaverine an inhibitor of smooth muscle.

Assays on the dose-response curves of $CaCl_2$. The preparations were kept in De Jalon solution until a stable baseline had been achieved, after which it was substituted by calcium-free Ringer hyperpotassic solution. After a period of 15–30 min, a cumulative dose-response curve of $CaCl_2$ was plotted up to a maximum response. These curves were repeated in the presence of different doses of the respective substances to be assayed (reticuline, verapamil and papaverine), placing them in the bath 5 min before starting the dose-response curve.

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Drug sources. Reticuline base was isolated from the alkaloid-enriched extract of *Laurobasidium lauri* (Geyler) Jülich (Exobasidiaceae), specimens were collected in the laurus forest of Porto Moniz (Madeira Island) during the summer of 1986. The material was authenticated by Prof. Amaral Franco of the Agronomy Institute of Lisbon and the reticuline was characterized by NMR spectroscopic techniques by Prof. Diaz Martin of the Organic Chemistry Department of the University of Salamanca.

The following agents were used: verapamil hydrochloride (Knoll-Made), papaverine hydrochloride (Sigma), calcium chloride (Sigma), and potassium chloride (Sigma).

The reticuline alkaloid was dissolved in aqueous phosphate buffer, pH 7·2, and nutrient solution was used as solvent for the other agents.

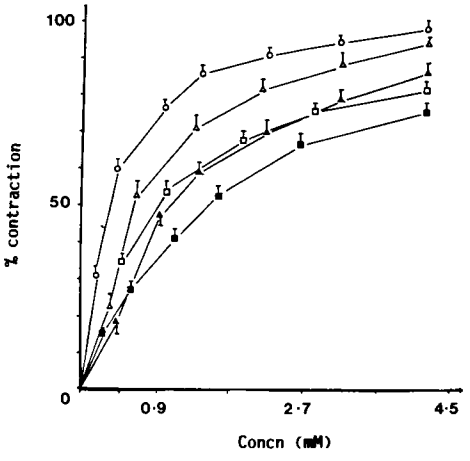

FIG. 1. Effect of: reticuline (△) $3{\cdot}0 \times 10^{-8}$ M, (▲) $3{\cdot}0 \times 10^{-7}$ M; and papaverine (□) $5{\cdot}3 \times 10^{-7}$ M, (■) $4{\cdot}4 \times 10^{-6}$ M on the mean cumulative concentration-response curves obtained to calcium chloride (○) control. Each point represents the mean +s.e.m. of 5 experiments.

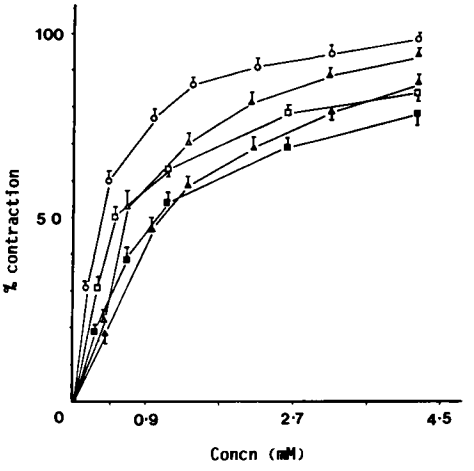

FIG. 2. Effect of: reticuline (△) $3{\cdot}0 \times 10^{-8}$ M, (▲) $3{\cdot}0 \times 10^{-7}$ M; and verapamil (□) $8{\cdot}5 \times 10^{-9}$ M, (■) $3{\cdot}4 \times 10^{-8}$ M on the mean cumulative concentration response curves obtained to calcium chloride (○) control. Each point represents the mean ±s.e.m. of 5 experiments.

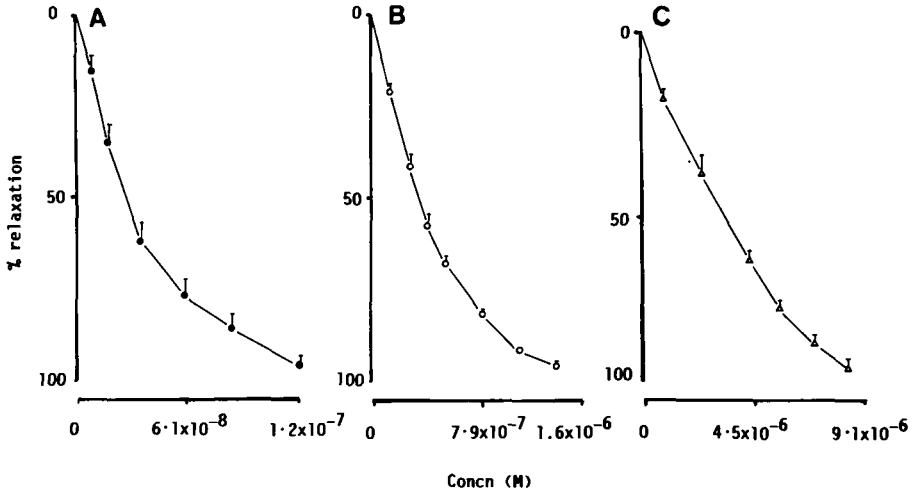

FIG. 3. Effect of didemethyl-t-butyl ICI 118,551 (▲,△) and 4D (●,○) on rabbit ciliary process (open symbols) and cardiac ventricle (closed symbols) isoprenaline-stimulated activated adenylate cyclase.

Results

Increasing cumulative doses of reticuline, added to the bath during the potassium-induced contraction plateau, led to a pronounced dose-dependent relaxation (Fig. 3C) similar to those observed with papaverine and verapamil (Fig. 3A, B) but less potent.

The concentrations of isolated reticuline assayed were seen to produce a shift in the dose-response curves of $CaCl_2$ towards zones of higher concentrations, giving rise to a significant increase in the efficacious concentration 50 (EC-50) and reducing the maximum effect (E_{max}). This is similar to what happens in the case of verapamil and papaverine (Table 1, Figs 1, 2).

Table 1. EC50 (mean, n = 5) and E_{max} (mean ± s.e.m, n = 5) values of calcium chloride in the presence of solution saline (control), reticuline base, verapamil hydrochloride, and papaverine hydrochloride.

		EC50 (M)	E_{max}
Control (M)		$4{\cdot}36 \times 10^{-4}$	100
Reticuline	$(3{\cdot}0 \times 10^{-8})$	$8{\cdot}25 \times 10^{-4}$	$93{\cdot}3 \pm 0{\cdot}5$
Reticuline	$(3{\cdot}0 \times 10^{-7})$	$9{\cdot}3 \times 10^{-4}$	$91{\cdot}2 \pm 1{\cdot}5$
Verapamil	$(8{\cdot}5 \times 10^{-9})$	$1{\cdot}06 \times 10^{-3}$	$90{\cdot}9 \pm 1{\cdot}6$
Verapamil	$(3{\cdot}4 \times 10^{-8})$	$1{\cdot}53 \times 10^{-3}$	$87{\cdot}6 \pm 1{\cdot}9$
Papaverine	$(5{\cdot}3 \times 10^{-7})$	$7{\cdot}10 \times 10^{-3}$	$93{\cdot}2 \pm 1{\cdot}3$
Papaverine	$(4{\cdot}4 \times 10^{-6})$	$1{\cdot}02 \times 10^{-3}$	$92{\cdot}1 \pm 2{\cdot}1$

Discussion

The results obtained with reticuline, papaverine and verapamil on potassium-depolarized uteri are similar and agree with those obtained with ketotifen and verapamil by other authors (Villar et al 1985; Lowe & Richardson 1980), who have proposed that the agents act as blockers to the entry of calcium from the extracellular milieu because in potassium depolarization a contraction occurs owing to the increase in intracellular calcium (Van Breemen et al 1978; Bolton 1979; Brading 1981).

Furthermore, the shift of the $CaCl_2$ curves towards zones of higher concentration, a fact demonstrated for verapamil (Advenier et al 1984; Villar et al 1985) and observed in the present study with reticuline, verapamil and papaverine, shows that these agents prevent the entry of calcium into the cell through voltage-dependent channels. This is because in the case of the $CaCl_2$ dose-response curves in hyperkalaemic serum and without calcium, a depolarization of the membrane occurs accompanied by an opening of the voltage-dependent calcium channels (Villar et al 1985). Accordingly the contraction is due to the calcium added, i.e. the entrance of calcium through the volage-dependent channels (Ichida et al 1984).

In the light of the present findings and those previously obtained (Sagredo et al., unpublished), the uterine inhibitory effect of reticuline is mainly related to a decrease in the concentration of cytosolic calcium available for contraction, perhaps affecting one or several mechanisms that participate in the movement of extra- or intracellular calcium.

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