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Effect of the Hydroalcoholic Extract of *Rauwolfia ligustrina* on Smooth and Cardiac Muscles In-vitro

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Abstract

The actions of the hydroalcoholic extract (HE) of *Rauwolfia ligustrina* (the whole plant) on agonist-induced contractions were analysed in the rat uterus and guinea-pig ileum and trachea, and also in rat atrium contracting spontaneously in-vitro.

The HE (100–400 $\mu g\ mL^{-1}$) caused concentration-dependent rightward shifts of the concentration–response curves and reduced the maximal contraction induced by oxytocin, bradykinin, angiotensin II, prostaglandin $F_{2\alpha}$ and acetylcholine in the rat uterus. The calculated mean IC50 values were: 300, 147, 158, 197 and 105 $\mu g\ mL^{-1}$, respectively. In the guinea-pig ileum the HE also caused graded displacement to the right of the concentration–response curves for bradykinin, histamine and carbachol, associated with pronounced inhibition of the agonist-induced maximal contractions. The calculated mean IC50 values were 165, 134 and 241 $\mu g\ mL^{-1}$, respectively. The HE (100–3000 $\mu g\ mL^{-1}$) caused graded relaxation (IC50 of 271 $\mu g\ mL^{-1}$) of strips of guinea-pig trachea precontracted with carbachol (0·2 μM). This effect was not infuenced by propranolol (1 μM), 3-isobutyl-1-methylxanthine (1 μM) or methylene blue (10 μM), but was significantly potentiated (1·5-to 3-fold) by indomethacin (3 μM) and forskolin (1 nM). In addition, N^G-monomethyl-L-arginine (L-NMMA, 100 nM) significantly reduced the HE-induced maximal relaxation, while indomethacin (3 μM) potentiated the HE response. Finally, the HE caused a concentration–dependent and long-lasting inotropic effect in the rat right atrium, contracting spontaneously with a mean EC50 value of 409 $\mu g\ mL^{-1}$.

It is suggested that the effects of the HE of *R. ligustrina* on smooth and cardiac muscles 'in-vitro' may result from its ability to interact, at least partially, with the cAMP pathway.

Rauwolfia ligustrina Roem. et Schult. (*Apocynaceae*), a reported toxic species, is popularly known in the north east of Brazil as 'Arrebenta-boi'. Preliminary phytochemical analysis carried out on this plant has revealed that *R. ligustrina* contains alkaloids, flavonoids and steroids (Agra & Barbosa-Filho 1990). Martinez et al (1992) have recently isolated eight alkaloids from the stem bark of this species; identified as tetrahydroalstonine, aricine, isoreserpiline, reserpinine, reserpiline, α-yohimbine, isoreserpinine and ajmaline.

Preliminary pharmacological study showed that the hydroalcoholic extract (HE) (70% v/v) of *R. ligustrina* produces concentration-dependent relaxation in rat aorta, precontracted with low (17·5 nM) and and high (80 nM) KCl concentrations, an effect which was insentitive to the ATP-sensitive Ca^{2+}-channel blocker glibenclamide (Medeiros et al 1994).

In the present study, we have investigated further the pharmacological actions of the HE extract of *R. ligustrina* (the whole plant) on agonist-induced contractions in several preparations.

Materials and Methods

Preparation of the extract

Botanical material was collected and classified by Dra. Maria de Fátima Agra (Departamento of Ciências Farmacêuticas e Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba, João Pessoa, Paraíba, Brazil). The whole plant material was dried at room temperature and extracted exhaustively with 70% ethanol/water (v/v) in a Soxhlet apparatus for 72 h. The solvent was evaporated to dryness in a rotary evaporator under a vacuum at 50°C and conserved at 4°C until use.

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Pharmacological procedures

Rat uterus. Preparations were obtained from female Wistar rats, 200–250 g, pretreated 24 h earlier with oestradiol benzoate (0·5 mg kg^{-1}, s.c.). Strips, 15–20 mm long were suspended in 5 mL of aerated De Jalon solution, composition (mM): NaCl 154; KCl 5·6; $CaCl_2$ 0·3; $MgCl_2$ 1·4; $NaHCO_3$ 1·7 and glucose 5·5, maintained at 30°C. Isotonic contractions were recorded by means of a light lever (6-fold amplification) inscribing on a kymograph under 1 g. After an equilibration period of 30–40 min, complete concentration–response curves for bradykinin, oxytocin, acetylcholine, prostaglandin $F_{2\alpha}$ and angiotensin II were obtained at 30-min intervals (Van Rossum 1963). Once the responses became reproducible, the preparations were exposed to increasing concentrations of HE from *R. ligustrina* (100–400 $\mu g\ mL^{-1}$) for 10 min, and the new concentration-response curves for the agonists were constructed in its presence. Different periods of incubation of the HE with the preparations (up to 60 min) were tested, and 10 min proved to be the best. The maximal contraction obtained for a given agonist in the second control concentration-response curve (before exposure to extract) was taken as the 100% response, and all subsequent responses were calculated as functions of

this value. Only one agonist was tested on each strip. To correct for spontaneous desensitization during the course of the experiments, separate analogous experiments were performed in tissues using only the vehicle used to dilute the extract or drugs.

Guinea-pig ileum. Strips of ileum 3 to 4 cm long were obtained from guinea-pigs of both sexes, 300–500 g, and were set up for recording of isotonic contractions in a 5-mL organ bath containing gassed (95% O_2+5% CO_2) Krebs-Henseleit solution (mM: NaCl 113; KCl 4·7; $CaCl_2$ 2·5; $NaHCO_3$ 25; $MgSO_4$ 1·1; KH_2PO_4 and glucose 11) at 37°C, under 1 g of load. After an equilibration period of 30–40 min, cumulative concentration-response curves for carbachol, bradykinin and histamine were constructed, first in the absence and after 30 min in the presence of HE from *R. ligustrina* (100–400 μg mL^{-1}) as described for the rat uterus.

Guinea-pig trachea. Guinea-pigs of either sex, 200–350 g, were killed by a blow on the head and were exsanguinated from carotid arteries. Usually, four transverse rings (3–4 mm wide) were obtained from each animal. The rings were opened and strips of 8–10 mm in length with intact epithelium were suspended in individual 5-mL jacketed organ baths containing Krebs-Henseleith solution at 37°C, pH 7.2, and gassed with 95% O_2 and 5% CO_2. After equilibration for at least 60 min, preparations were contracted by addition of carbachol (0.2 μM), and once the response plateaued (which usually took about 15 min), cumulative inhibitory concentration-response curves were obtained for the HE (10–3000 μg mL^{-1}) in the absence or presence of the following drugs: methylene blue (10 μM, an inhibitor of guanylate cyclase), propranolol (1 μM, a non-selective β-adrenoceptor antagonist), 3-isobutyl-1-methylxanthine (IBMX, 1 μM, a phosphodiesterase inhibitor), indomethacin (3 μM, a cyclo-oxygenase inhibitor), L-NMMA (100 μM, an inhibitor of nitric oxide biosynthesis) or forskolin (1 nM, an activator of adenylate cyclase) incubated with the preparations 20 min beforehand. The relaxation caused by the HE in the absence or presence of antagonists was determined in mg of tension, relative to carbachol-induced tonus.

Rat right atrium. Right atria were excised from female Wistar rats, 200–250 g, set up in gassed (95% O_2 and 5% CO_2) Krebs-Henseleit solution at 37°C and submitted to a resting tension of 1 g. Following 60 min of equilibration and when the spontaneous isometric contraction became stable, a cumulative concentration–response curve for HE of *R. ligustrina* (100–3000 μg mL^{-1}) was obtained. Responses were presented as percentage of increase of basal spontaneous contraction.

Drugs

The following drugs were used: acetylcholine iodide, propranolol hydrochoride, bradykinin, prostaglandin $F_{2\alpha}$, oestradiol benzoate, 3-isobutyl-1-methylxanthine, histamine chloride, indomethacin, angiotensin II, forskolin, N^G-monomethyl-L-

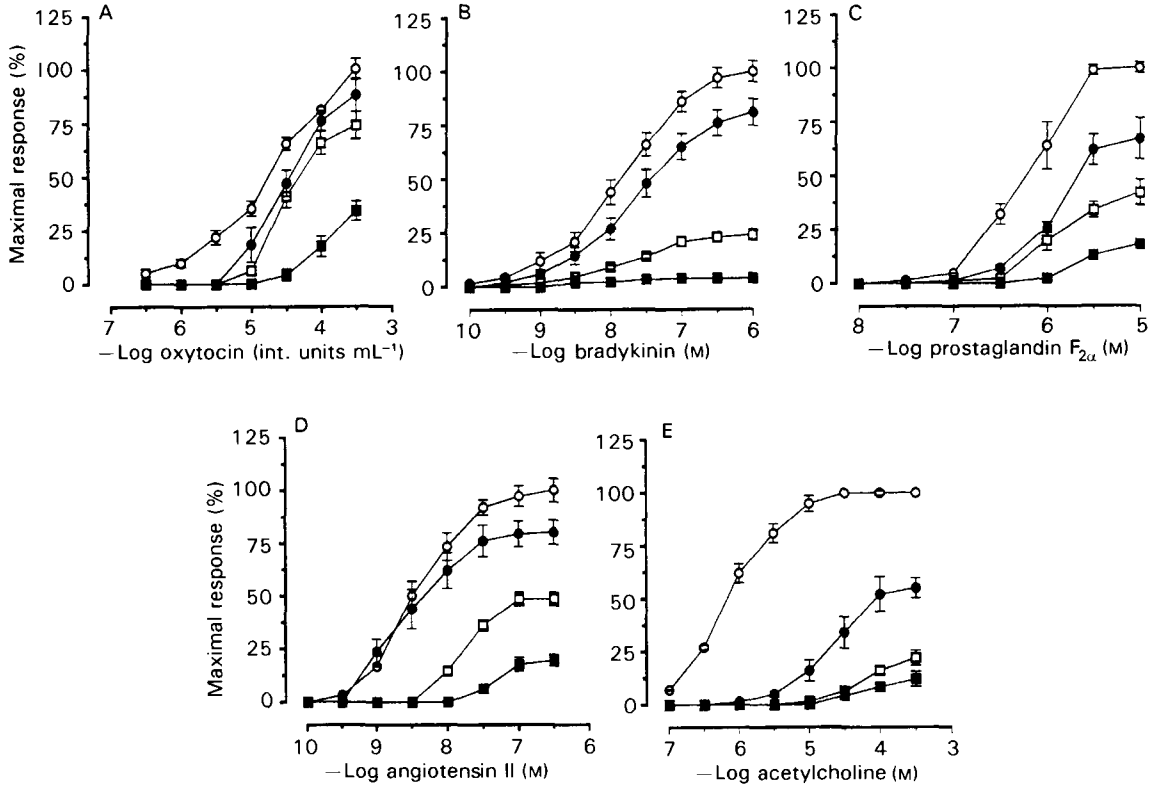

FIG. 1. Effect of hydroalcoholic extract (HE) of *Rauwolfia ligustrina* on contractile cumulative concentration-response curves caused by oxytocin (A), bradykinin (B), prostaglandin $F_{2\alpha}$ (C), angiotensin II (D) and acetylcholine (E) in the rat isolated uterus. Control responses (○) and responses obtained in the presence of increasing concentration of the HE (μg mL^{-1}): ● 100; □ 200 and ■ 400. Values are mean ± s.e.m. of 5 or 6 experiments.

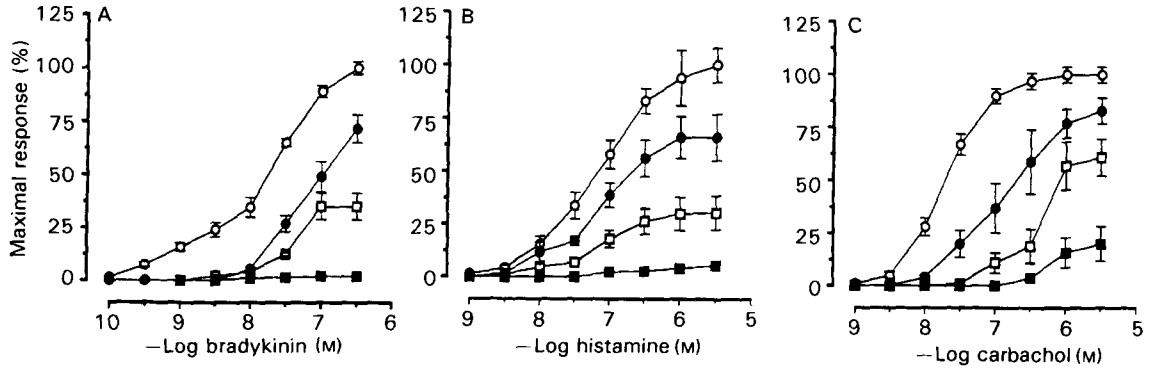

FIG. 2. Effect of hydroalcoholic extract (HE) of *Rauwolfia ligustrina* on the contractile cumulative concentration–response curves caused by bradykinin (A), histamine (B) and carbachol (C) in the guinea-pig isolated ileum. Control responses (○) and responses obtained in the presence of increasing concentrations of HE ($\mu g\ mL^{-1}$): ● 100; □ 200 and ■ 400. Values are mean ± s.e.m. of 5 or 6 experiments.

arginine (L-NMMA, all from Sigma Chemical, St. Louis, USA) and oxytocin (Syntocinon, Sandoz, Brazil). All the reagents used were of analytical grade (Merck) and solutions and drugs were prepared daily in distilled and deionized water. Most drugs were stored as 10 to 100 mM stock solution at −20°C and were diluted to the desired concentrations just before use. Control preparations were exposed to the vehicle used to dilute the drugs.

Statistical analysis

Results are expressed as the means ± s.e.m. The IC50 values were determined from the individual concentration-response curves by using the least squares method. *P* values less than 0.05 were considered as indicative of significance.

Results

Preincubation of the rat uterine smooth muscle with the HE of *R. ligustrina* (100–400 $\mu g\ mL^{-1}$) 10 min before agonist additions resulted in a concentration–dependent reduction of agonist-induced contractions allied to marked rightward displacement of the oxytocin, bradykinin, prostaglandin $F_{2\alpha}$, angiotensin II and acetylcholine-induced contractions (Fig. 1, A-E). The calculated mean IC50 values were: 300, 147, 158, 197 and 105 $\mu g\ mL^{-1}$ for oxytocin, bradykinin, prostaglandin $F_{2\alpha}$ and angiotensin II, respectively.

When added to the guinea-pig isolated ileum, the HE (100–400 $\mu g\ mL^{-1}$) caused concentration–dependent displacements to the right of the bradykinin, histamine and carbachol concentration-response curves, accompanied by marked inhibition of the maximal responses to the agonists (Fig. 2, A-C). The calculated mean IC50 values for these effects were: 165, 134 and 241 $\mu g\ mL^{-1}$ for bradykinin, histamine and carbachol, respectively. Cumulative addition of HE of *R. ligustrina* (100–3000 $\mu g\ mL^{-1}$) to the guinea-pig trachea strips precontracted with carbachol (0.2 μM) resulted in graded relaxation. The incubation of the preparations with indomethacin (3 μM) or forskolin (1 nM) significantly shifted to the left (1.47 and 2.97 fold) the relaxant concentration response curve for HE (mean IC50 values were: controls 673 and 271 vs 456 and 91 $\mu g\ mL^{-1}$ for treated preparations, respectively) (Fig. 3, A and B). In addition, indomethacin, but not forskolin, significantly potentiated the maximal relaxation response caused by the HE (control response 934 ± 47 vs 1189 ± 59 mg for treated preparations) (Fig. 3A).

In contrast, the nitric oxide synthase inhibitor L-NMMA (100 μM) significantly inhibited the relaxation induced by the HE (Fig. 3C). However, the maximal relaxant response caused by HE of *R. ligustrina* (3000 $\mu g\ mL^{-1}$) was not influenced significantly by incubation of preparations with propranolol (1 μM, Fig. 3D), methylene blue (10 μM, Fig. 3E) or with IBMX (1 μM, Fig. 3F).

Addition of increasing concentrations of the HE of *R. ligustrina* (100–3000 $\mu g\ mL^{-1}$) to the rat isolated atrium, contracting spontaneously, produced concentration-dependent and long-lasting positive inotropic effect which was resistant to sucessive washouts of the tissues, with death of tissues (Fig. 4). The calculated mean EC50 for this effect was 409 $\mu g\ mL^{-1}$.

Discussion

The in-vitro pharmacological results presented in this study demonstrate that the HE extract of *R. ligustrina* produced concentration–dependent, reversible and equipotent inhibition of contractions induced by several neurotransmitters. Its action involves a non-competitive mechanism of action, because the inhibitory effect caused by the HE in relation to the contractile responses elicited by all studied agonists in both preparations was not overcome, in spite of the increase of the bath concentration of the agonists. Furthermore, the HE of *R. ligustrina* caused a concentration–dependent relaxation of guinea-pig trachea precontracted with carbachol, and produced graded and long-lasting but irreversible positive inotropic action of the rat isolated right atrium contracting spontaneously.

Activation of adenylate cyclase and accumulation of cAMP is the major signaling pathway underlying agonist-mediated relaxation in smooth muscles, including those in airways, through activation of both protein kinase A and G. Although the precise mechanism by which the extract of *R. ligustrina* induces inhibition of agonist–mediated contraction in rat uterus and guinea-pig ileum remains unknown, our results provide some support to indicate that accumulation of cAMP seems to be involved. This view is substantiated by the results showing that its relaxant effect in guinea-pig trachea precontracted with carbachol was significantly potentiated by forskolin, a direct activator of adenylate cyclase. The view that the HE of *R.*

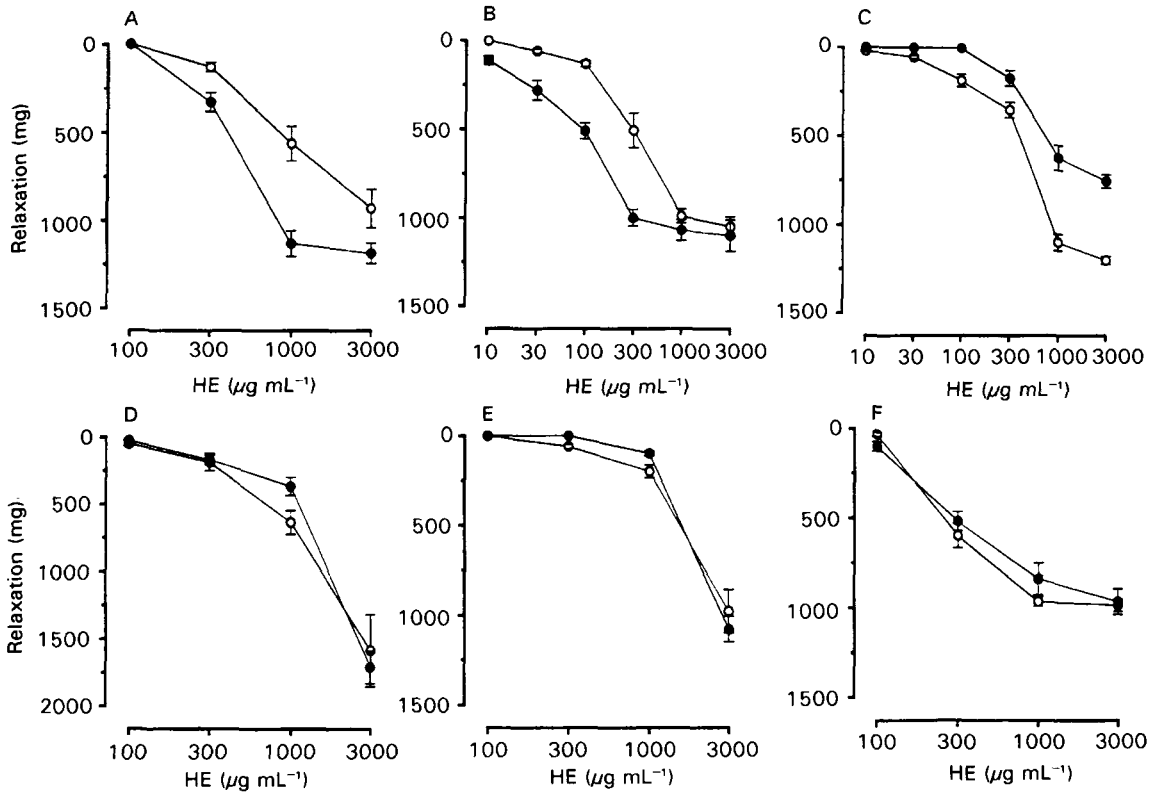

FIG. 3. Relaxant effect caused by the hydroalcoholic extract (HE) of *Rauwolfia ligustrina* in the guinea-pig isolated trachea pre-contracted with carbachol (0.2 μM) Control responses (○) and results obtained in the presence (●) of indomethacin (A, 3 μM), forskolin (B, 1 nM), N^G-monomethyl-L-arginine (C, 100 nM), propranolol (D, 1 μM), methylene blue (E, 10 μM) or 3-isobutyl-1-methylxanthine (F, 1 μM). Values are mean ± s.e.m. of 4 or 5 experiments.

ligustrina produced concentration–dependent and long-lasting positive inotropic effect in the rat isolated right atrium futher substantiated our notion that the active principle(s) present in this plant is acting through cAMP accumulation. Surprisingly, prior incubation of guinea-pig trachea with 3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor, failed to potentiate significantly the relaxation caused by HE. The reason for this discrepancy is still unclear, and was not investigated in more detail in the present study.

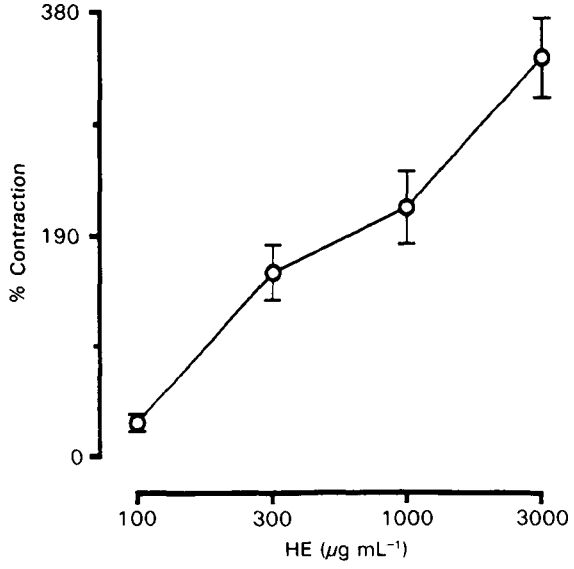

FIG. 4. Effect of the hydroalcoholic extract (HE) of *Rauwolfia ligustrina* on the rat isolated right atrium contracting spontaneously. Values are mean ± s.e.m. of 6 experiments.

The relaxation induced by HE of *R. ligustrina* in guinea-pig trachea is unlikely to involve interaction with β-adrenoceptors, as propranolol did not influence its relaxant effect. However, the fact that indomethacin significantly potentiated the relaxation induced by HE of *R. ligustrina* suggests that the cyclooxygenase metabolites derived from the arachidonic acid pathway may have some role in modulating its relaxant response. Furthermore, the nitric oxide synthase inhibitor L-NMMA significantly inhibited the maximal relaxation response induced by the HE, suggesting that its effect involves, at least partially, the release of nitric oxide or related substance. Interestingly, previous incubation of preparations with methylene blue, an inhibitor of soluble guanylate cyclase activated by nitric oxide (Gruetter et al 1981), at a concentration known to inhibit the relaxation mediated through cGMP in response to nitric oxide in vascular and non vascular smooth muscles had no significant effect on the vasorelaxation response induced by the HE of *R. ligustrina*. Such results suggest that although the cGMP-dependent protein kinase seems to be involved in control of airway smooth muscle tone, and taking into account the fact the nitric oxide inhibitor NMMA was able to inhibit part of the relaxation induced by the HE of *R. ligustrina*, the accumulation of cGMP nevertheless seems unlikely to participate in its relaxant effect.

Acknowledgements

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