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Effect of a crude extract of *Mandevilla velutina* on contractions induced by bradykinin and [des-Arg^9]-bradykinin in isolated vessels of the rabbit

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1 The effect of a crude aqueous/alcoholic extract of *Mandevilla velutina* on the contractile responses induced by bradykinin (Bk), [des-Arg^9]-Bk and noradrenaline (NA) in rings of arterial and venous rabbit vessels was analysed.

2 The contractile responses induced by Bk, [des-Arg^9]-Bk differed between the rings of the aorta, jugular and mesenteric veins. Rings of the aorta and mesenteric vein were stimulated by both peptides, but were more sensitive to [des-Arg^9]-Bk. The jugular vein was not only more sensitive to Bk but also did not respond to [des-Arg^9]-Bk, even in the presence of a high concentration of this peptide.

3 Pre-incubation for 20 min with the crude extract of *Mandevilla velutina* rhizomes (1 mg ml^{-1}) antagonized the contractions produced by both peptides. This blockade was surmountable by about a 10–30 fold increase in the concentration of both Bk and [des-Arg^9]-Bk. In the aorta and mesenteric vein the NA-induced contractions were not affected by 1 mg ml^{-1} of the extract.

4 Bk (1 to 100 nM) caused a concentration-related contractile response in rings of the rabbit jugular vein. Incubation for 20 min with the crude extract (0.25–1 mg ml^{-1}) caused a concentration-dependent displacement to the right of the Bk concentration-response curves and depressed the maximal response.

5 The onset of action of the crude extract was rapid and was reversible after intermittent washing of the preparations for 30–60 min.

6 These findings confirm and extend our previous work and indicate that the crude extract of *Mandevilla velutina* selectively antagonizes the action of Bk and related peptides on both B_1- and B_2-receptors present in rabbit vascular muscle.

Introduction

Recent studies on the structure-activity relationships of bradykinin (Bk) and related peptides indicated that the biological effects induced by these agents may involve at least two different type of receptors (Regoli *et al.*, 1977; 1981; Barabé *et al.*, 1977; 1979; Innis *et al.*, 1981; Gaudreau *et al.*, 1981; Fredrick *et al.*, 1984; 1985; for reviews see Regoli & Barabé, 1980; Regoli, 1982; 1984).

Recently, we have demonstrated that an aqueous/alcoholic extract of *Mandevilla velutina* rhizomes (Apocynaceae) antagonizes selectively the Bk-induced contractions in rat uterine muscle (Calixto *et al.*, 1985a). Since we have proposed that the active principle(s) present in this extract may be interfering with the Bk receptor, it became of interest to determine whether the extract of *Mandevilla velutina* interacts preferentially with one subtype of Bk receptor.

In the present study, we investigated the effect of *Mandevilla velutina* extract on the contractile response of rings of rabbit aorta, and mesenteric vein (contain B_1-receptors) and of jugular vein (B_2-receptors), to Bk and the octapeptide [des-Arg^9]-Bk. We have also compared the selectivity of the crude extract against noradrenaline-induced contractions in these preparations.

Methods

Preparation of crude extract

The crude aqueous/alcoholic extract of *Mandevilla velutina* rhizomes was prepared as described previously (Calixto *et al.*, 1985a). Thawed rhizomes were

minced and extracted with 50% ethanol-water in the proportion 1:3 (w/v), stirred mechanically at room temperature for 24 h and passed through filter paper. The extract was dessicated and suspended in 0.9% w/v NaCl solution to the desired concentration.

Preparations of rabbit isolated vessels

Rabbits of either sex weighing 1.5–2.5 kg were killed by a blow on the head. The thoracic segment of the aorta and the jugular and mesenteric veins were carefully isolated. Four millimetre long transverse rings of each vessel were mounted in a 10 ml jacketed organ bath containing Krebs-Henseleit solution maintained at 37°C, pH 7.2 and gassed continuously with 95% O_2 and 5% CO_2, according to Furchgott & Jawadzki (1980). The Krebs solution had the following composition (mM): NaCl 113, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.1, KH_2PO_4 1.1 and glucose 11. The preparations were initially allowed to equilibrate for at least 2 h under a resting tension of 2 g and 1 g for the aorta and veins, respectively. Isometric tension changes were measured by means of a force transducer F-60 (Narco-Byosystems) with a continuous tension of 1 g and 0.5 g for artery and veins, respectively.

Simple or cumulative concentration-response curves were obtained at 20 and 60 min intervals respectively, for Bk, [des-Arg^9]-Bk, and noradrenaline. Once the contractile responses were stabilized different concentrations of the crude aqueous/alcoholic extract of *mandevilla velutina* (0.25–2 mg ml^{-1}) were added to the bath and left in contact with the tissue for 20 min. Then a second simple or cumulative concentration-response curve was obtained for the different agonists. The sensitivity was evaluated at the ED_{50} level. Control experiments for noradrenaline, Bk and [des-Arg^9]-Bk were performed in separate vessels in the absence of the crude extract. The maximal contraction in the first concentration-response curves was taken as 100%, and all subsequent contractions calculated as a % of this value.

Drugs

Bradykinin triacetate, [des-Arg^9]-Bk (synthesized by Dr A.C.M. Paiva in the Department of Biophysics, Escola Paulista de Medicina, São Paulo, Brazil) and noradrenaline bitartrate (Sigma) were dissolved in an aqueous solution and kept at −4°C. These drugs were diluted just before use in aqueous solution to the desired concentration, except noradrenaline which was dissolved in 0.9% w/v NaCl solution containing ascorbic acid (50 μg ml^{-1}), in order to avoid oxidation.

Results

The contractile response of rings of the aorta, jugular and mesenteric veins of the rabbit to noradrenaline, Bk and [des-Arg^9]-Bk in the presence or absence of crude extract are shown in Figures 1, 2 and 3. The responses to Bk and to the octapeptide [des-Arg^9]-Bk differed between these preparations. Aortic rings were stimulated by both peptides, but this preparation was more sensitive to [des-Arg^9]-Bk (Figure 1). In contrast, rings of the rabbit jugular vein not only were more sensitive to Bk but also did not develop any contraction to even high concentrations of [des-Arg^9]-Bk (Figure 2). The responses of rings of the rabbit mesenteric vein to Bk and to [des-Arg^9]-Bk were very similar to those obtained in the rings of thoracic aorta (Figure 3). In these vessels, noradrenaline induced a rapid phasic contraction while Bk and [des-Arg^9]-Bk produced a tonic response that was reproducible when repeated at 20 min intervals. Pre-incubation for 20 min with the crude aqueous/alcoholic extract of *Mandevilla velutina* rhizomes (1 mg ml^{-1}) antagonized the contractile response induced by both Bk and [des-Arg^9]-Bk in these preparations. This blockade was surmountable by increasing the concentration of these agonists by about a 10–30 fold. On the other hand, the contractile responses to noradrenaline were not affected by the extract, except in the jugular vein where the contractile response to noradrenaline was partially depressed, at 1 mg ml^{-1} of the extract. As found earlier in the rat uterus (Calixto *et al.*, 1985), the onset of the anti-Bk activity was rapid (equilibration in about 10 min) and was reversed after intermitent washing of the preparations for 30–60 min (results not shown).

Bk (1–100 nM) caused dose-related contractions in the rings of jugular vein, with a maximal response of 2.63 ± 0.18 g of tension and an ED_{50} (95% confidence limits) of 41.9 (25.0–70.3) nM. As shown in Figure 4 the crude extract (0.25–1 mg ml^{-1}) caused a concentration-related displacement to the right of the Bk concentration-response curve and depressed the maximal response to about 70% of the control value at 1 mg ml^{-1}.

In the preparations studied, no change in sensitivity to the peptides during the incubation period was observed in the absence of the extract.

Noradrenaline (0.01 to 30 μM) caused a dose-related contraction in the rings of rabbit aorta with a maximal response of 5.48 ± 0.25 g of tension and a ED_{50} (95% confidence limits) of 0.33 (0.21–0.52 μM). Pre-incubation of the preparations with the crude extract of *M. velutina* (1 to 2 mg ml^{-1}) for 20 min, did not significantly change either the sensitivity or the maximal response to noradrenaline (Figure 5).

Discussion

The present findings confirm and extend our previous work (Calixto *et al.*, 1985a) indicating that a crude

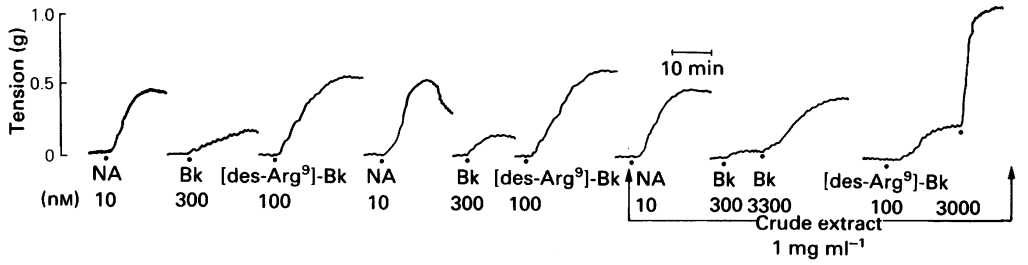

Figure 1 Typical isometric responses to noradrenaline (NA), bradykinin (Bk) and to the octapeptide [des-Arg^9]-Bk of a ring of rabbit aorta in the absence and presence of the crude aqueous alcoholic extract of *Mandevilla velutina* rhizomes (1 mg ml^{-1}). The numbers indicate the final drug concentration. Representative record of at least 7 experiments.

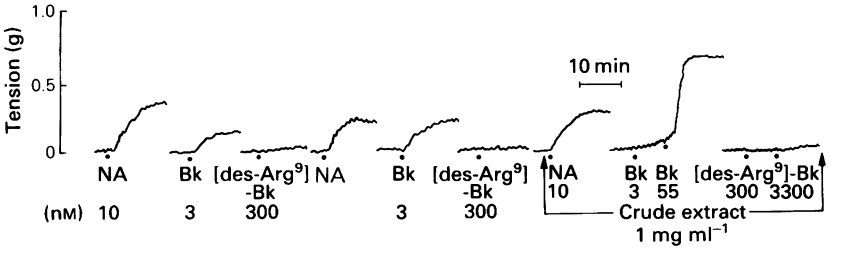

Figure 2 Typical isometric responses to noradrenaline (NA), bradykinin (Bk) and [des-Arg^9]-Bk of a ring of rabbit jugular vein in the absence and presence of the crude extract of *Mandevilla velutina*. Representative responses of 7 experiments.

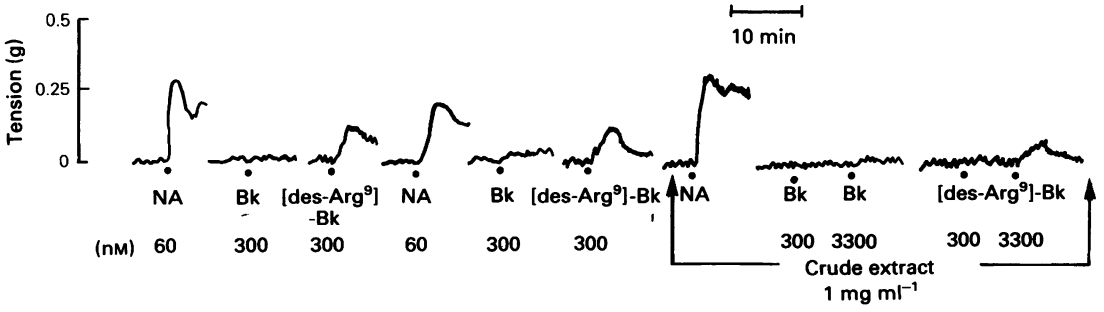

Figure 3 Typical isometric responses to noradrenaline (NA), bradykinin (Bk) and [des-Arg^9]-Bk of a ring of rabbit mesenteric vein in the absence and presence of the crude extract of *Mandevilla velutina*. Representative responses of at least 6 experiments.

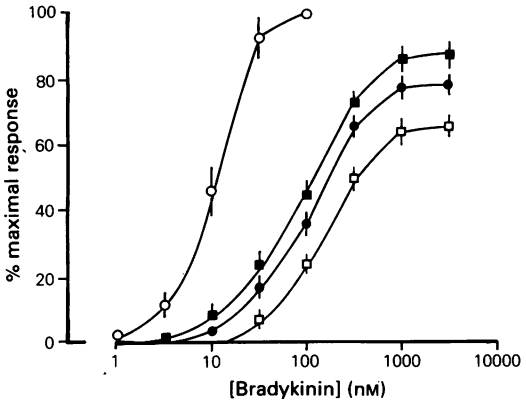

Figure 4 Mean concentration-response curves for the effects of bradykinin on rings of the rabbit jugular vein in the absence (control curve, O) and presence of increasing concentrations of the crude extract of *Mandevilla velutina* rhizomes: 0.25 (■), 0.5 (●), 1.0 (□) mg ml^{-1}. Each point represents the mean of 6 to 8 experiments and the vertical lines indicate the s.e.mean.

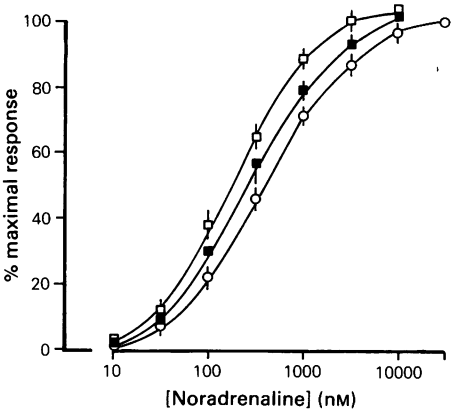

Figure 5 Mean concentration-response curves for the effects of noradrenaline in rings of the rabbit aorta in the absence (control curve, O) and presence of increasing concentrations of crude extract of *Mandevilla velutina* rhizomes: 1 (●) and 2 (□) mg ml^{-1}. Each point represents the mean of 6 to 7 experiments and the vertical lines indicate the s.e.mean.

extract of *Mandevilla velutina* antagonizes selectively the contractions induced by Bk and related peptides mediated by both B_1- and B_2-receptors. These results also indicate that there are some differences between the action of the crude extract on B_2-receptors present in uterine muscle and those on the rabbit jugular vein described here. While in the uterus no change in the maximal response to Bk was observed, even in presence of high concentrations of this extract (2 mg ml^{-1}), a small but significant (about 30%) depression of the maximal contraction to Bk was observed in rings of the jugular vein at 1 mg ml^{-1} (Figure 4). Thus, the antagonism displayed by the crude extract against Bk in the jugular vein could not be considered to be competitive and the estimation of a pA_2 value was not possible. The differences in the effects of the extract in the two preparations may be related, at least in part, to the existence of several compounds that may act differently in these two preparations.

The absence of a change in sensitivity to Bk and the octapeptide [des-Arg^9]-Bk in the rabbit isolated aorta during the incubation period *in vitro* is not in accordance with the observations of other laboratories (Regoli *et al.*, 1977; 1978; Barabé *et al.*, 1979). These discrepancies may be related to differences in the methodology employed, since in the present study the vascular reactivity *in vitro* was analysed in the presence of endothelial cells.

Considering that both B_1- and B_2-receptors may be involved in the vascular action of kinins in some physiological and pathological conditions, including the inflammatory process (Regoli & Barbaré, 1980; Marceau *et al.*, 1983; Regoli, 1984), these results could support, at least in part, the folk medicinal use of the infusion of alcoholic extracts of rhizomes from *Mandevilla velutina*. In fact, unpublished results indicate that the crude extract of this plant is active when given orally (100 to 200 mg kg^{-1} body weight) in blocking the paw oedema induced by carrageenin and other agents, and the *Brothops jararaca*-induced increase in cutaneous capillary permeability, in the rat. Recently, several studies have indicated that the increase in vascular permeability, the paw oedema caused by Bk and the relaxant effect of kinins in isolated arteries are mediated by the B_2-receptor type (Marceau *et al.*, 1980; Whalley *et al.*, 1984). In addition, the extract also antagonized the endothelial-dependent vasodilator action of Bk and Lys-Bk, when it was added during the course of the relaxation response of rings of the dog femoral and mesenteric arteries precontracted with noradrenaline (Calixto *et al.*, 1985b).

In conclusion, these findings provide further evidence for the antagonistic activity of the *M. velutina* extract against Bk and show that the crude extract selectively antagonizes the action of Bk and related peptides on both B_1- and B_2-receptors present in rabbit vascular smooth muscle.

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