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The selective antagonism of bradykinin action on rat isolated uterus by crude *Mandevilla velutina* extract

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A crude aqueous alcoholic extract of *Mandevilla velutina* (Apocynaceae) rhizomes produced a concentration-dependent displacement to the right of the concentration-response curve to bradykinin (Bk) in the isolated uterus of the rat. Schild analysis of the data revealed a linear relationship ($r = 0.99$) and yielded a pA_2 value of $3.3 + 0.08$ ($-\log g\,ml^{-1}$) but the slope differed significantly from unity. The anti-Bk action was of rapid onset and was reversible upon washout. Contractions induced by acetylcholine, oxytocin, angiotensin II and $BaCl_2$ were unaffected by the extract. This represents the first report of a selective Bk antagonist of plant origin. The isolation of the active principle(s) may result in a useful pharmacological tool for elucidating the physiological and physiopathological roles of endogenous Bk.

Introduction The folk medicine in certain regions of central-western Brazil prescribes the use of infusions or alcoholic extracts of rhizomes from *Mandevilla velutina* (Apocynaceae) for the treatment of venomous snake bites, including those of *Bothrops jararaca*. In view of the bradykinin (Bk)-releasing properties (Rocha e Silva *et al.*, 1949) and Bk-potentiating factors (Ferreira, 1965) of *Bothrops jararaca* venom, we have attempted to investigate this plant for anti-Bk activity using the isolated uterus of the rat. This preparation possibly represents a useful bioassay for the determination of selective anti-Bk activity since it responds to a wide variety of agonists besides Bk and does not contain kininase II (Stewart, 1979).

Methods Rhizomes from *Mandevilla velutina* (variety glabra) were collected in the State of Minas Gerais, Brazil, and stored at −20°C until use. The extraction procedure closely resembled that employed in folk medicine. Thawed rhizomes were minced and extracted with 50% ethanol-water in the proportion 1:3 (w/v), mechanically stirred at room temperature for 24 h following filtration through filter paper. The extract was dessicated and resuspended in 0.9% w/v NaCl solution to the desired concentration.

Uterine fragments were obtained from virgin Wistar rats (180–250 g), 24 h after treatment with oestradiol benzoate (0.5 mg kg^{-1}, s.c.) and mounted for recording of isotonic contractions as previously described (Calixto & Loch, 1985). After an equilibration period of 30–40 min, successive cumulative or non-cumulative concentration-response curves for Bk, acetylcholine (ACh), oxytocin (Ot), angiotensin II (AII), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and $BaCl_2$ were constructed at 30 min intervals. Once reproducible curves to a given agonist were obtained, the crude extract of *Mandevilla velutina* (0.5 to 4 mg ml^{-1}) was added to the bath and a last curve constructed in its presence. Each preparation was exposed to only one agonist. The sensitivitiy to all agonists was evaluated at the EC_{50} level. The pA_2 value fo the extract against Bk (as $-\log g\,ml^{-1}$) was calculated by graphical interpolation according to the method of Arunlakshana & Schild (1959).

Acetylcholine chloride, prostaglandin $F_{2\alpha}$, angiotensin II (all from Sigma), barium chloride (Merck), oxytocin (Syntocinon, Sandoz, São Paulo) and bradykinin (synthesized by Dr A.C.M. Paiva in the Department of Biophysics, Escola Paulista de Medicina, São Paulo, Brazil) were dissolved in aqueous solution. Oestradiol benzoate and indomethacin (Sigma) were diluted in peanut oil (1 mg ml^{-1}) and absolute ethanol, respectively.

Results Preparations exposed to the extract (0.5–4 mg ml^{-1}) for 20 min showed a concentration-dependent shift to the right of the concentration-response curve to Bk (0.1–1000 nM) (Figure 1a); the crude aqueous/alcoholic extract (0.01–10 mg kg^{-1}) did not affect uterine tone. The Schild plot of the data revealed a linear relationship ($r = 0.99$) and yielded a pA_2 value of 3.3 ± 0.08 (Figure 1a, inset) but the slope differed significantly from unity (-1.87 ± 0.2). The onset of anti-Bk activity was rapid (equilibration in about 10 min) and was reversed completely after intermittent washings of the preparation for 30–60 min. Addition of indomethacin (1–10 μM) to the bathing solution did not influence the inhibition of Bk action by the extract.

Uterine responses to Ot (0.01–30 iu ml^{-1}), ACh (0.01–100 μM), AII (1–100 nM) and $BaCl_2$ (1–30 nM)

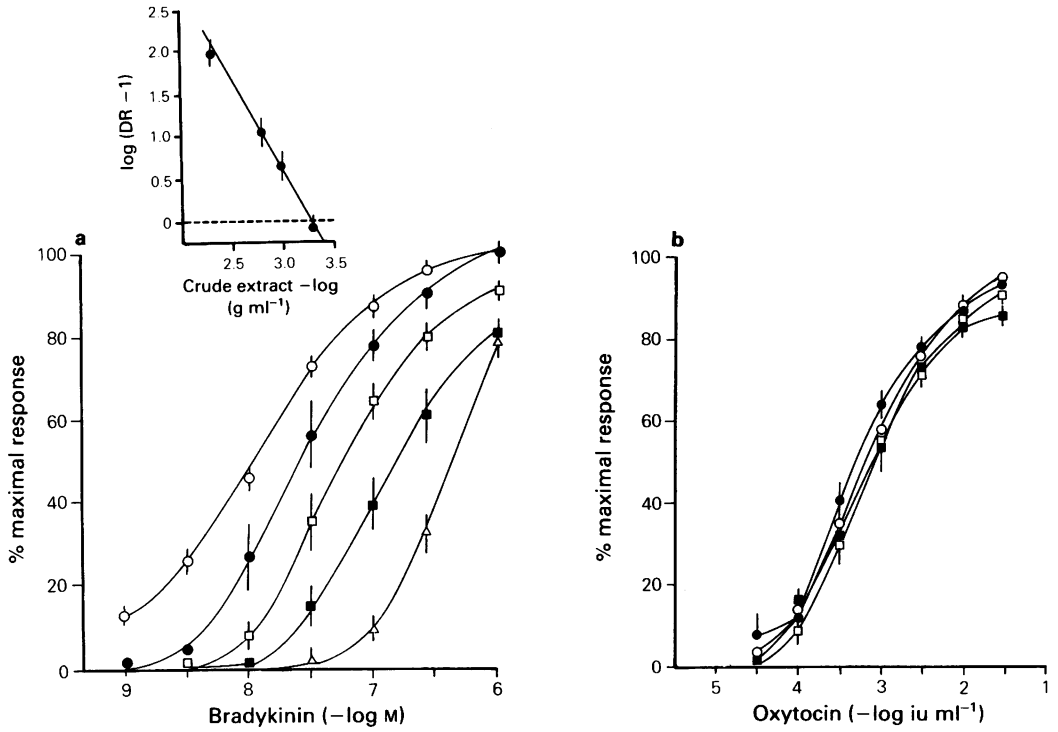

Figure 1 Mean concentration-response curves obtained for bradykinin (a) and oxytocin (b) in the isolated uterus of the rat. Control curve (○) or in presence of increasing concentrations of crude extract of *Mandevilla velutina* rhizomes (mg ml^{-1}): 0.5 (●); 1 (□); 2 (■) and 4 (△). *Inset*: Schild plot for crude extract of *Mandevilla velutina* as an antagonist of bradykinin-induced contraction of the isolated uterus of the rat. The apparent pA_2 values were determined by interpolation using regression analysis. Each point represents the mean of 8 to 12 experiments and the vertical lines the s.e.mean.

were not affected by the extract (0.5–4 mg ml^{-1}). Only the data on Ot are shown (Figure 1b). In contrast, the extract shifted the curve for $PGF_{2\alpha}$ to the left at 1 mg ml^{-1} (about 2 fold) but reduced the maximum response to this agonist at 2 mg ml^{-1}.

Discussion The present findings clearly show that the crude extract of *Mandevilla velutina* rhizomes inhibits Bk-induced contractions of the isolated uterus of the rat. This effect is dose-dependent and appears to be highly selective for Bk. The highest concentration of extract tested (4 mg ml^{-1}) produced a rightward displacement of the Bk curve of about 60 fold but did not significantly alter the sensitivitiy or maximal-response to ACh, Ot, AII or $BaCl_2$. However, the extract did attenuate the maximal response to $PGF_{2\alpha}$ at 2 mg ml^{-1}.

It has been suggested that prostaglandins may mediate part the action of Bk on the rat uterus (Terragno *et al.*, 1974). Thus, the hypothesis was raised that the extract may interfere with prostaglandin biosynthesis and/or action. This appears not to be the case since indomethacin (up to 10 μM) did not modify the anti-Bk action of the extract and, surprisingly, low doses (1 mg ml^{-1}) of the extract sensitized the uterus to $PGF_{2\alpha}$. Also, it is not yet possible to ascribe a simple competitive action of the extract at Bk receptors because the slope of the regression line obtained in the Schild plot against Bk differed significantly from unity. However, this may indicate that the crude extract contains several active principles with different affinities for the Bk receptor. Indeed, preliminary phytochemical analysis indicates the presence of at least three different compounds (possibly terpenes) with varying anti-Bk activity in the extract (manuscript in preparation).

The present study provides experimental support for the popular use of this plant in the treatment of venomous snake bites and represents the first demonstration of a selective Bk antagonist from natural sources. The future characterization of the active

principle(s) may result in the development of new pharmacological tools which could be useful not only in investigating the physiological and physiopathological roles of endogenous Bk (for reviews see Rocha e Silva, 1970; Colman & Wong, 1979) but also in the management of disease states involving Bk and/or related kinins. We are now actively engaged in this task.

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