Br. J. Pharmac. (1987), **91**, 199–204

Kinin antagonist activity of compounds from *Mandevilla velutina* in the rat isolated uterus

[1]*João B. Calixto, *Mauro Nicolau, **Moacir G. Pizzolatti & **Rosendo A. Yunes

Departments of *Pharmacology CCB and **Chemistry CFM, Universidade Federal de Santa Catarina, 88000 – Florianópolis, SC, Brazil

1 The effect of four semi-purified compounds obtained from *Mandevilla velutina* crude extract by silica gel chromatography fractionization were analysed for their inhibitory effects on uterine contractions induced by bradykinin (BK), lysylbradykinin (L-BK), acetylcholine (ACh) and oxytocin, *in vitro*.

2 None of the compounds tested affected uterine tone. Pre-incubation for 20 min with fraction 12 (20 to 80 $\mu g\,ml^{-1}$), isolated from *M. velutina* produced a parallel and concentration-dependent displacement to the right of the concentration-response curves for BK and L-BK (1 to 1000 nM). Schild plots of these data were linear (correlation close to unity), and yielded a nominal pA_2 value (as $g\,ml^{-1}$) of 5.1 and 4.9, respectively, and the values of the slopes were not significantly different from unity. Fraction 11 (10 to 40 $\mu g\,ml^{-1}$) also produced a parallel and concentration-dependent displacement to the right of the BK concentration-response curve. The Schild plot gave a mean pA_2 value ($g\,ml^{-1}$) of 5.4 and a slope not significantly different from unity.

3 Fraction 12 did not influence the uterine contractile responses induced by ACh (0.1 to 100 μM) and oxytocin (0.01 to 30 miu ml^{-1}) at concentrations less than 80 $\mu g\,ml^{-1}$.

4 Fraction 16 (20 to 80 $\mu g\,ml^{-1}$) antagonized the action of BK only at concentrations greater than 40 $\mu g\,ml^{-1}$, whereas fraction 5 (20 to 80 $\mu g\,ml^{-1}$) was completely inactive against BK-induced responses.

5 As observed previously with the crude extract, the onset of the BK antagonistic action of these compounds was rapid and completely reversible following intermittent washing of the preparation for 30–60 min.

6 These results indicate the existence of at least two compounds in the crude extract of *Mandevilla velutina* that act as competitive and selective kinin antagonists on the isolated uterus of the rat.

Introduction

Since the discovery of bradykinin (BK) release from plasma globulin by snake venoms and by trypsin (Rocha e Silva *et al.*, 1949), there is increasing evidence indicating that BK and the decapeptide kallidin (Lys-bradykinin, L-BK) may participate in several physiological and pathological processes (Manning *et al.*, 1982; for reviews, see Rocha e Silva, 1963; 1970; Kellermeyer & Graham, 1968; Colman & Wong, 1979; Regoli & Barabé, 1980). The interest in kinin action increased after the demonstration of a BK-potentiating effect in the *Bothrops jararaca* venom (Ferreira, 1965; Ferreira *et al.*, 1970). However, the knowledge of the exact physiological and pathological role of kinins in many biological systems still remains unknown because no selective and competitive kinin antagonist exists. Although several hundred analogues of BK have been synthesized and analysed pharmacologically, only a few peptides have been shown to block competitively the action of kinin in some biological models (Regoli & Barabé, 1980; Varvek & Stewart, 1985; Regoli *et al.*, 1986).

Recently, we have demonstrated that the crude aqueous/alcoholic extract of *Mandevilla velutina*, a plant used in certain regions of Brazil for treatment of venomous snake bites, including those of *Bothrops jararaca*, and also used for the treatment of inflammation, selectively antagonized, in a concentration-dependent and reversible manner, BK-induced responses in the rat isolated uterus (Calixto *et al.*, 1985a). The crude extract of this plant was also active in

[1] Author for correspondence.

antagonizing BK- and L-BK-induced responses in other smooth muscles such as guinea-pig ileum, rat duodenum and dog mesenteric and femoral arteries (Calixto *et al.*, 1985b). In addition, the crude extract selectively antagonized the contractile effect of BK mediated by both B_1- and B_2-receptors in rabbit vascular smooth muscles (Calixto & Yunes, 1986).

The purpose of the present work was, therefore, to analyse the effect of four fractions isolated from *Mandevilla velutina* upon the contractile responses induced by BK and L-BK in the rat uterus, in order to characterize the anti-BK compound(s) present in this plant. We also tested the effect of fraction 12 upon acetylcholine (ACh)- and oxytocin-induced contractions in these preparations in order to verify its selectivity against BK. The results indicate that at least two fractions isolated from the crude extract of *Mandevilla velutina* contained selective and competitive antagonists of kinins in the rat isolated uterus.

Methods

Procedure for isolation of the semipurified compounds

The rhizomes of *Mandevilla velutina* were ground into small pieces and extracted with ethyl acetate. The extract was filtered and evaporated to yield a brown powder that accounts for 9% of the rhizomes. The extract was fractionated by silica gel (Merck 7734) column chromatography with a methylene chloride system containing increasing amounts of ethyl acetate. Fractions were collected and monitored by thin layer chromatography t.l.c., Merck Silica Gel G eluted with toluene-EtOAc-MeOH (55:45:5) and visualized with short and long wavelength u.v. light or with anisaldehyde-AcOH-MeOH-H_2SO_4 (0:5:10:85:5) spray.

From 88 fractions those that showed the same t.l.c. spots were placed together giving twenty new fractions. From these fractions 11 and 12 exhibited BK antagonistic activity showing two and three different compounds, respectively. Details of the isolation procedure and physical and chemical characterization of these compounds will be published elsewhere.

Evaluation of pharmacological activity

Uterine preparations were obtained from virgin Wistar rats (180–250 g) kept in a room of controlled temperature (22 ± 1°C) and illumination (12 h on and 12 h off). The animals were treated with oestradiol benzoate (0.5 mg kg^{-1}, s.c.) 24 h before the experiments. Uterine strips (1.5 cm long) free from adhering tissues were suspended in 5 ml organ baths containing 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 and continuously bubbled with air. Isotonic contractions were measured under a resting load of 1 g and recorded by means of a light lever (six fold amplification) writing on a kymograph.

Following the equilibration period (30–40 min), successive cumulative concentration-response curves for BK, L-BK, ACh and oxytocin were constructed at 30 min intervals (van Rossum, 1963). Once reproducible curves to a given agonist had been obtained, different concentrations of crude extract (0.5 to 2 mg l^{-1}) or the fractions isolated from *M. velutina* (10 to 80 μg ml^{-1}) were added to the bath and left in contact with the tissue for 20 min. Then a second

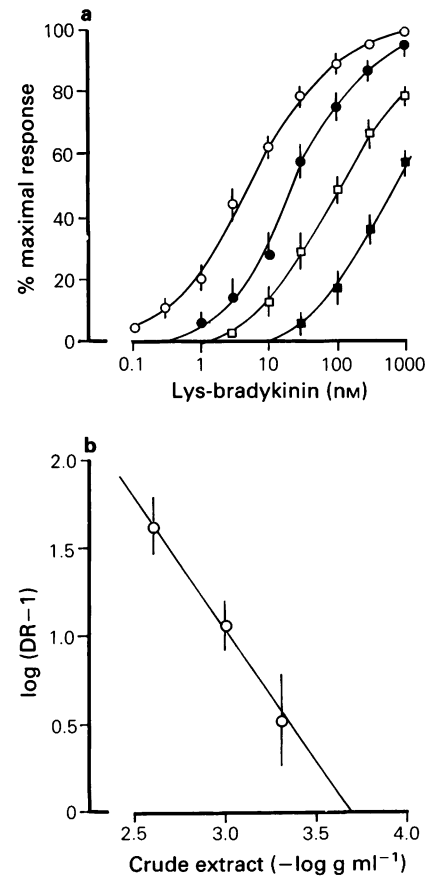

Figure 1 (a) Mean concentration-response curves for lysyl-bradykinin in the isolated uterus of the rat. Control curve (○) and curves in presence of increasing concentrations of crude extract of *M. velutina* rhizomes (mg ml^{-1}): 0.5 (●), 1 (□) and 2 (■). (b) Schild plot for crude extract as antagonist of Lys-bradykinin-induced contractions in the isolated uteri of the rat. The nominal pA_2 values are presented in Table 1 and were determined by interpolation using regression analysis. Each point represents the mean of 5 to 6 experiments and vertical lines indicate s.e. mean .

Table 1 The mean nominal pA_2 values (as g ml^{-1}) for the antagonistic effects of crude extract and fractions 11 and 12, isolated from *Mandevilla velutina*, against bradykinin and lysyl-bradykinin-induced contractions in the isolated uterus of the rat

Antagonist	*Agonist*	*pA_2*[a]	*Slope*[a]	*Correlation*
Crude extract	Bradykinin	3.2 (2.9–3.6)*	1.8 (1.5–2.3)	0.92 ± 0.002
(n = 5–7)	Lysyl-bradykinin	3.5 (3.2–3.7)	1.7 (1.5–2.1)	0.98 ± 0.02
Fraction 12	Bradykinin	5.1 (4.8–5.4)	1.2 (1.0–1.3)	0.99 ± 0.005
(n = 5)	Lysyl-bradykinin	4.9 (4.6–5.4)	1.3 (1.0–1.7)	0.96 ± 0.01
Fraction 11	Bradykinin	5.4 (4.3–5.8)	1.1 (0.6–1.6)	0.97 ± 0.02
(n = 5)				

[a] Numbers in parentheses represent 95% confidence limits.
[b] Data shown are mean ± s.e. mean.
* Calixto *et al.*, 1985a.

cumulative concentration-response curve was obtained. Each fraction was tested in separate strips and control experiments were performed in the absence of these compounds. The nominal pA_2 values (as log g ml^{-1}) for the crude extract, and for the compounds isolated from *M. velutina*, against BK and L-BK-induced contractions were calculated by graphical interpolation according to the method of Arunlakshana & Schild (1959).

Statistical analysis

The results are presented, when appropriate, as mean ± s.e.mean. Statistical significance of differences between the means was assessed by use of Student's t test for unpaired data.

Drugs

The following drugs were used: acetylcholine iodide (Sigma), oxytocin (Syntocinon, Sandoz, São Paulo, Brazil), bradykinin and lysyl-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 (Sigma) and fractions from *M. velutina* were dissolved in peanut oil (1 mg mg^{-1}) and absolute ethanol (20 mg ml^{-1}), respectively. The final bath concentration of ethanol was lower than 0.01%.

Results

As found previously in the rat uterine muscle for BK (Calixto *et al.*, 1985a) the aqueous/alcoholic crude extract of *M. velutina* (0.5 to 2 mg ml^{-1}) also caused a parallel and concentration-dependent displacement to the right of the L-BK (1 to 1000 nM) concentration-response curve (Figure 1). Schild plot regression lines for these data (Figure 1b) yielded a straight line and gave an apparent pA_2 value (as log g ml^{-1}) not significantly different ($P > 0.05$) from those obtained for BK (Table 1). However, for both BK and L-BK the mean slope values obtained differed significantly from unity (Table 1). As shown in Figure 2, pre-incubation for 20 min of the preparations with fraction 12 (20 to 80 μg ml^{-1}) isolated from *M. velutina* rhizomes

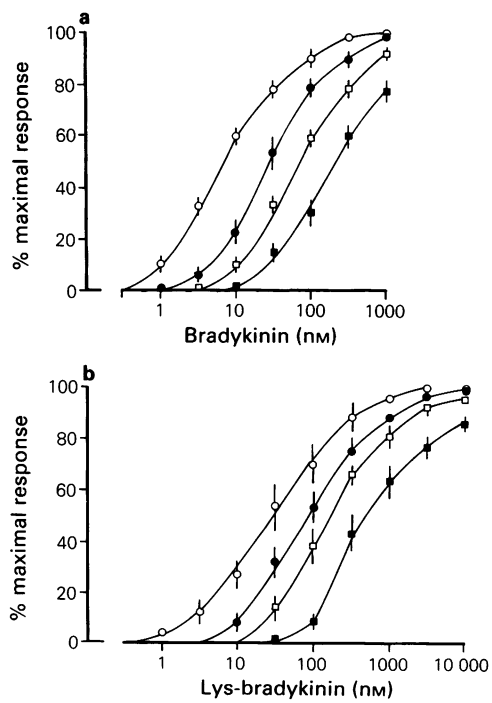

Figure 2 Mean concentration-response curves for (a) bradykinin and (b) lysyl-bradykinin in the isolated uterus of the rat. Control responses (○) and responses in presence of increasing concentrations of fraction 12 isolated from *M. velutina* rhizomes (μg ml^{-1}): 20 (●), 40 (□) and 80 (■). Each point represents the mean of 5 experiments and vertical lines indicate s.e. mean.

produced a parallel and concentration-dependent shift to the right of the concentration-response curves for both BK and L-BK. The Schild plot of these data revealed a linear relationship (correlation close to one) and yielded similar mean pA_2 values for BK and L-BK with the slopes of Schild regression analyses not significantly different from unity (Figure 3 and Table 1). The concentration-response curve for BK was also displaced to the right in a parallel and concentration-dependent manner by fraction 11 (10 to 40 $\mu g\,ml^{-1}$) isolated from *M. velutina* (Figure 4a). The Schild plot for these data was linear ($r = 0.97$) and showed that this fraction also behaved as a simple and competitive antagonist of BK, since the calculated slope was not significantly different from unity (Figure 4b and Table 1).

Fraction 12 failed to influence the uterine contractile response induced by acetylcholine (0.1 to 100 μM) and oxytocin (0.01 to 30 miu ml^{-1}) at concentrations less than 80 $\mu g\,ml^{-1}$ (Figure 5a and b). Incubation of the preparation with fraction 16 (20 to 80 $\mu g\,ml^{-1}$) for 20 min produced only a weak effect on the BK dose-response curve at concentrations greater than 40 $\mu g\,ml^{-1}$, whereas in the same experimental conditions fraction 5 (20 to 80 $\mu g\,ml^{-1}$) was completely inactive-against the response to BK (Figure 6 a and b). All compounds were devoid of agonistic activity in rat uterine muscle. As shown previously with the crude extract, in rat uterine muscle and in the isolated vessels of the rabbit (Calixto *et al.*, 1985a; Calixto & Yunes, 1986), the onset of the BK antagonistic activity of these compounds isolated from *M. velutina* was rapid and was completely reversible following intermittent washing of the preparation for 30–60 min.

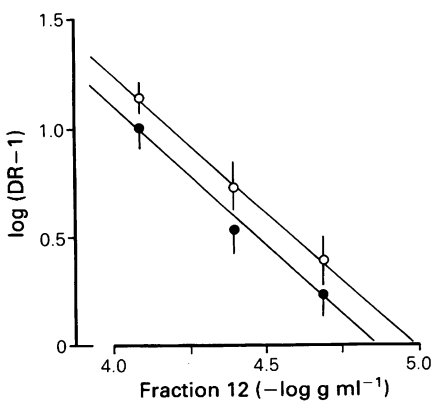

Figure 3 Schild plots for fraction 12 isolated from *M. velutina* rhizomes as antagonist of bradykinin (○)- and lysyl-bradykinin (●)-induced contractions in the isolated uterus of the rat. The nominal pA_2 values are presented in Table 1. Each point represents the mean of 5 experiments and vertical lines indicate s.e. mean.

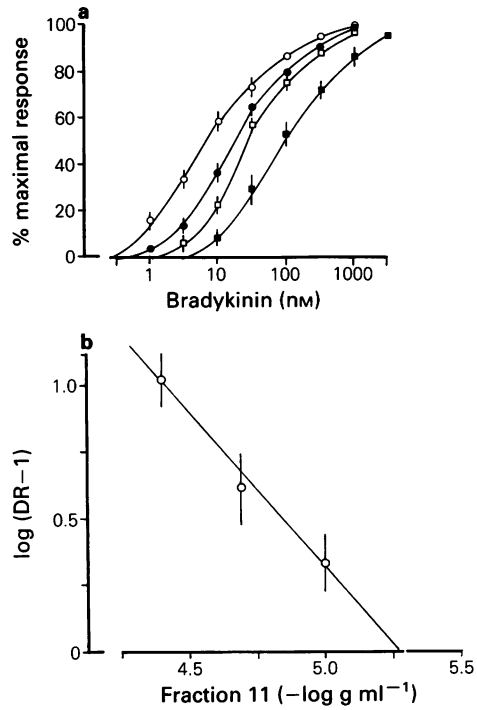

Figure 4 (a) Mean concentration-response curves for bradykinin in the isolated uterus of the rat. Control responses (○) and responses in the presence of increasing concentrations of fraction 11 isolated from *M. velutina* ($\mu g\,ml^{-1}$): 10 (●), 20 (□) and 40 (■). (b) Schild plot for fraction 11 as antagonist of bradykinin-induced contractions in the isolated uterus of the rat. The nominal pA_2 values are presented in Table 1. Each point represents the mean of 5 experiments and vertical lines indicate s.e. mean.

Discussions

Consistent with our previous observations (Calixto *et al.*, 1985a) the present findings indicate that the crude extract of *M. velutina* selectively antagonizes, in a concentration-dependent manner, the contractions induced by BK and L-BK in the rat uterine muscle (pA_2 values as log g ml^{-1}, 3.2 and 3.5 respectively). For both peptides, the antagonistic effect of the crude extract cannot be considered as simple competition, since the slopes of the Schild regression lines were significantly different from unity (1.8 and 1.7 for BK and L-BK, respectively), suggesting that the crude extract may contain more than one active principle with different affinities for kinin receptors.

In fact the fractionation of crude extract from *M. velutina* rhizomes by silica gel chromatography allowed the isolation of at least two fractions that are

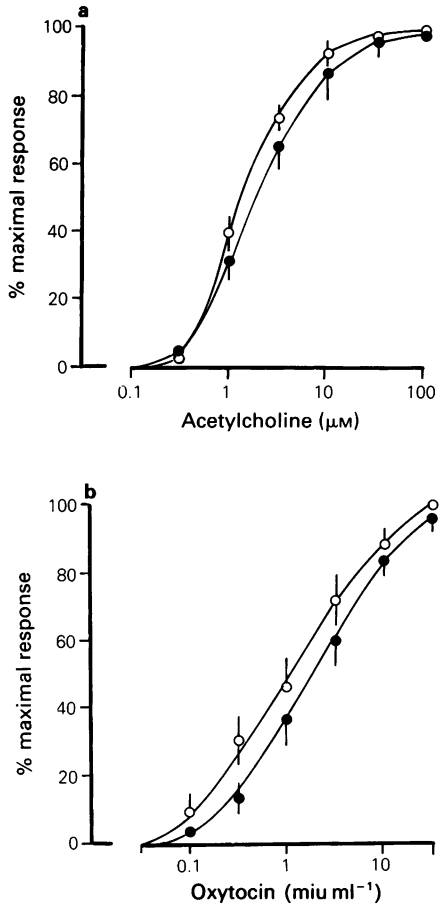

Figure 5 Mean concentration-response curves for (a) acetylcholine and (b) oxytocin in the isolated uterus of the rat. Control responses (O) and responses in presence of fraction 12 isolated from *M. velutina*, 40 μg ml⁻ (●). Each point represents the mean of 4 to 6 experiments and vertical lines indicate s.e. mean.

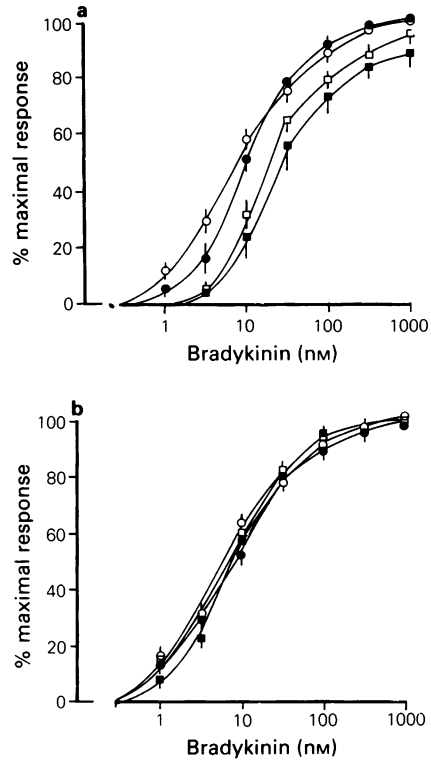

Figure 6 Mean concentration-response curves for bradykinin in the isolated uterus of the rat. Control responses (O) and responses in presence of fraction 16 (a) and fraction 5 (b) isolated from *M. velutina* rhizomes (μg ml⁻¹): 20 (●), 40 (□) and 80 (■). Each point represents the mean of 4 to 5 experiments and vertical lines indicate s.e. mean.

selective and competitive antagonists of BK and L-BK actions in the rat uterus. In contrast to the crude extract, both fractions (11 and 12) antagonized the kinin action on the uterus of the rat with a slope of Schild regression line not different from unity, suggesting that such compounds were interacting at the actual kinin receptor. Thus, the antagonistic effect may be considered as simple in nature, satisfying the criteria needed for receptor classification (Tallarida *et al.*, 1979; Kenakin, 1982). Fractions 11 and 12 isolated from *M. velutina* were about 79 and 158 fold more potent in antagonizing the uterine contractile response induced by BK than the crude extract, while fraction 12 was about 20 fold more potent than the crude extract in antagonizing the contractile response induced by L-BK (Table 1). The similar apparent pA_2 values obtained for the crude extract (3.2 and 3.5) and also for compound 12 isolated from *M. velutina* (5.1 and 4.9) against BK and L-BK-induced contractile responses, together with the similar slopes of Schild regression analyses, indicate that the two peptides may be interacting at the same receptor in the rat uterus. The antagonistic effect of these compounds was reversible, clearly concentration-dependent, and selective for kinins (BK and L-BK), since the sensitivity and the maximal response for acetylcholine and oxytocin concentration-response curves were not affected by the compound in fraction 12, at a concentration less than 80 μg ml^{-1}. Concentrations above 80 μg ml^{-1} of fraction 12 reduced the maximal response to both agonists, an effect that may be partially related to the vehicle used to dilute this compound.

Since we have recently demonstrated that the crude

extract of *M. velutina* selectively antagonizes the action of BK and [des-Arg^9]-BK on both B_1- and B_2-receptors present in rabbit vascular smooth muscles (Calixto & Yunes, 1986), it is possible that these fractions now isolated from this plant, are active against both sub-types of kinin receptor. In addition the crude extract was also effective in antagonizing the endothelial-dependent vasodilatation induced by BK and L-BK in ring preparations of the dog femoral and mesenteric arteries, that had been precontracted with noradrenaline (Calixto *et al.*, 1985b). It is possible that the two fractions isolated from this plant may be responsible for such effects.

In conclusion, these results indicate the existence of at least two fractions in the crude extract of *M. velutina* that act as simple competitive and selective kinin antagonists on the isolated uterus of the rat. Such compounds may be useful tools for evaluating the participation of BK and related kinins in both physiological and pathological processes.

The authors are grateful to Dr Gustavo Ballejo for helpful criticisms of the manuscript and Miss Adenir Pereira, Mrs Elza A. Bernardini for technical assistance. This work was supported by FINEP (grant no. 43850105) and CNPq.

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

CALIXTO, J.B., NICOLAU, M. & YUNES, R.A. (1985a). The selective antagonism of bradykinin action on isolated rat uterus by crude *Mandevilla velutina* extract. *Br. J. Pharmac.*, **85**, 729–731.

CALIXTO, J.B., NICOLAU, M. & YUNES, R.A. (1985b). A selective antagonist of bradykinin action from crude extract of *Mandevilla velutina*. Part II. Effect on non-uterine smooth muscle. *Brazilian J. med. biol. Res.*, **18**, 5–6, 729A.

CALIXTO, J.B. & YUNES, R.A. (1986). Effect of a crude extract of *Mandevilla velutina* on bradykinin and (des-Arg^9)-BK-induced contraction of isolated rabbit vessels. *Br. J. Pharmac.*, **85**, 937–941.

COLMAN, R.W. & WONG, P.Y. (1979). Kallikrein-kinin system in pathologic conditions. In *Handbook of Experimental Pharmacology*, Vol. XXV (Supplement), ed. Erdos, E.G. pp. 569–596. Berlin: Springer-Verlag.

FERREIRA, S.H. (1965). A bradykinin potentiating factor (BPF) present in the venom of *Bothrops jararaca*. *Br. J. Pharmac.*, **24**, 163–169.

FERREIRA, S.H., BARTLETT, O.C. & GREENE, L.J. (1970). Isolation of bradykinin potentiating peptides from *Bothrops jararaca* venom. *Biochemistry*, **9** (13), 2583–2592.

KELLERMEYER, R.W. & GRAHAM, R.C. (1968). Kinins. Possible physiologic and pathological roles in man. *New Engl. J. Med.*, **279**, 754–866.

KENAKIN, T. (1982). The Schild regression in the process of receptor classification. *Can. J. Physiol. Pharmac.*, **60**, 249–265.

MANNING, D.C., SNYDER, S.H., KACHUR, J.F., MILLER, R.J. & FIELD, M. (1982). Bradykinin receptor-mediated chloride secretion in intestinal function. *Nature*, **299**, 256–259.

REGOLI, D. & BARABÉ, J. (1980). Pharmacology of bradykinin and related kinins. *Pharmac. Rev.*, **32**, 1–46.

REGOLI, D., DRAPEAN, G., ROVERO, P., DION, S., D'ORLEANS-JUSTE, P. & BARABÉ, J. (1986). The actions of kinin antagonists on B_1 and B_2 receptors. *Eur. J. Pharmac.*, **123**, 61–65.

ROCHA E SILVA, M. (1963). The physiological significance of bradykinin. *A. New York Acad. Sci.*, **104**, 190–202.

ROCHA E SILVA, M. (1970). Participation of bradykinin and related kinins in physiological and pathological phenomena. In *Kinin Hormones with Special Reference to Bradykinin and Related Kinins*. ed. Kugekmass, L.N., pp. 170–216. Springfield: Charles C. Thomas.

ROCHA E SILVA, M., BERALDO, W.T. & ROSENFIELD, G. (1949). Bradykinin, a hypotensive and smooth muscle stimulating factor released from plasma by snake venoms and by trypsin. *Am. J. Physiol.*, **156**, 261–273.

TALLARIDA, R.J., COWAN, A. & ADLER, M.W. (1979). pA_2 and receptor differentiation a statistical analysis of a competitive antagonism. *Life Sci.*, **25**, 637–654.

VAN ROSSUN, J.M. (1963). Cumulative dose-response curves. Technique for the making of dose response curves in isolated organs and the evaluation of drug parameters. *Arch. int. Pharmacodyn. Ther.*, **143**, 299–330.

VAVREK, R.J. & STEWART, J.M. (1985). Competitive antagonists of bradykinin. *Peptides*, **6**, 161–164.

(Received November 7, 1986.
Accepted January 8, 1987.)