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The competitive antagonistic effect of compounds from *Mandevilla velutina* on kinin-induced contractions of rat uterus and guinea-pig ileum *in vitro*

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1 Pure non-peptide compounds obtained in crystal form from the crude extract of the plant *Mandevilla velutina* (Apocynaceae) were analysed for their antagonistic effects on rat uterine and guinea-pig smooth muscle contractions induced by bradykinin (Bk), lisyl-bradykinin (L-Bk), acetylcholine (ACh), oxytocin and histamine, *in vitro*.

2 Pre-incubation of rat uterine muscle with compounds MV 8608, MV 8609, MV 8610, MV 8611 and MV 8612 (5 to 40 $\mu g\,ml^{-1}$) caused parallel and concentration-dependent rightward displacements of the Bk concentration-response curves (1 to 1000 nM). Schild plots of these data were linear (correlation close to 1) and yielded nominal pA_2 values ($g\,ml^{-1}$) of 5.7, 5.6, 5.4, 5.7 and 5.3, respectively. Compounds MV 8608, MV 8611 and MV 8612 (5 to 20 $\mu g\,ml^{-1}$) also caused concentration-dependent and parallel displacements to the right of the concentration-response curve to L-Bk (1 to 10,000 nM). The Schild plots were linear and furnished nominal pA_2 values ($g\,ml^{-1}$) of 5.4, 5.8 and 5.1, respectively. With the exception of the antagonist effect of compound MV 8606 against Bk-induced contraction, all compounds behaved as simple competitive kinin antagonists since the calculated slopes were not different from unity.

3 In the guinea-pig ileum, both MV 8608 and MV 8612 (5 to 20 $\mu g\,ml^{-1}$), produced concentration-dependent rightward displacements of the concentration-response curve to Bk (0.1 to 1000 nM) when the experiments were performed in the presence but not in the absence of atropine (2.5 μM). However, in contrast to the result obtained in the rat uterus, compound MV 8608 also caused a significant reduction of the maximal response to Bk. The Schild plot for compound MV 8612 was linear (correlation close to unity) and furnished a nominal pA_2 value ($g\,ml^{-1}$) of 5.3 and a slope not different from unity.

4 In rat uterine muscle, compounds MV 8608 and MV 8612 at concentrations producing marked rightward displacements of the kinin concentration-response curves (10 and 20 $\mu g\,ml^{-1}$), did not influence the uterine contractile response to oxytocin or ACh, indicating some selectivity towards kinin receptors. Similarly, compound MV 8612 (10 and 20 $\mu g\,ml^{-1}$) did not interfere with the sensitivities or the maximal responses to ACh and histamine in the guinea-pig ileum, whereas compound MV 8608 (10 and 20 $\mu g\,ml^{-1}$) caused a slight reduction of ACh- and histamine-induced maximal contractions, allied to decrease of the sensitivity to histamine at concentrations of 20 $\mu g\,ml^{-1}$ or more.

5 These results extend our previous data, indicating the existence of several non-peptide compounds in the crude extract of *Mandevilla velutina* that act as simple, competitive, selective and reversible kinin receptor antagonists in the rat isolated uterus and guinea-pig ileum smooth muscle.

Introduction

Mandevilla velutina (Apocynaceae) is a plant native to Brazil which is used as a folk medicine in certain regions for the treatment of inflammatory states and of venomous snake bites, including those caused by *Bothrops jararaca*. Although there were no references to this plant species in the specialized literature, possibly because its use is geographically quite restricted, we have recently reported that the crude

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extract of *M. velutina* selectively and competitively inhibits bradykinin (Bk)-induced contractions of the isolated uterus of the rat (Calixto *et al.*, 1985a). Subsequently, we have also shown that the extract selectively inhibits responses to Bk and related kinins acting on both B_1- and B_2-receptors in vascular and non-vascular smooth muscle preparations (Calixto *et al.*, 1985b; 1987a, b; Calixto & Yunes, 1986). Moreover, when given orally, the crude *M. velutina* extract inhibits paw oedema caused by intraplantar injection of carrageenan and other phlogistic agents in mice and rats as well as pleurisy and increases in vascular permeability caused by Bk and *B. jararaca* venom in mice and rats, respectively (Calixto *et al.*, 1986; Nicolau *et al.*, 1986). *B. jararaca* venom is known for its Bk-releasing and Bk-potentiating factors (Rocha e Silva *et al.*, 1949; Ferreira 1965). Therefore, it is probable that the effects of *M. velutina* extract observed *in vivo* could be partially ascribed to an anti-Bk action.

More recently (Calixto *et al.*, 1987a), we demonstrated that fractionation of the crude extract yields at least two active fractions containing a mixture of compounds which act as selective and simple competitive antagonists at Bk-receptors of the rat uterus. The present work examines further the effects of five compounds isolated from *M. velutina* in crystalized form on the rat isolated uterus and the guinea-pig ileum. The results clearly show that this plant species contains several non-peptide substances capable of antagonizing Bk-induced responses in a selective and in a competitive manner.

Methods

Procedure for isolation of pure compounds from M. velutina

Isolation and purification of compounds exhibiting kinin antagonistic activity was performed as described previously (Calixto *et al.*, 1987a). Briefly, fraction 11, which contained two substances, was chromatographed over preparative t.l.c. silica gel 60 F_{254} plates. The plates were developed with toluene:ethylacetate:methanol (55:45:5). Zones were detected by u.v. and eluted with MeOH, and pure compounds MV 8608 and MV 8609 were obtained.

The three compounds present in fraction 12, were first separated by analytical t.l.c. with silica gel 6 F_{254} plates 0.2 mm thick eluted with hexane:diisopropylether:acetone (4:4:3). Then fraction 12 was subjected to preparative t.l.c. with silica gel 60 F_{254} plates 2.0 mm thick, eluted six times with hexane:diisopropylether:acetone (4:4:3). This procedure allowed the isolation of three compounds, named MV 8610, MV 8611 and MV 8612.

These anti-Bk compounds, which appear, from preliminary spectral analysis, to be terpene glycosides, represent only minor constituents of the plant, and furnished yields between 0.001 to 0.0001% of the crude plant extract. Moreover, the amount of each one of these compounds appeared to vary depending on the sample of botanical material used. More details on the isolation procedures and the physical and chemical characterization of these compounds will be published elsewhere.

Evaluation of pharmacological activity

Rat uterus Uterine strips 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 muscle strips (15 mm long) free from adhering tissues were mounted in 5 ml jacketed 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 recorded under a resting load of 1 g by means of a light lever (six fold amplification) writing on a kymograph.

Following an equilibration period of 30–40 min, cumulative concentration-response curves for bradykinin (Bk), lysyl-bradykinin (L-Bk), acetylcholine (ACh), and oxytocin were constructed at 30 min intervals (van Rossum 1963). After the concentration-response curves for a given agonist became stable (generally after 2 consecutive curves), different concentrations of the several compounds isolated from *M. velutina* (5 to 40 $\mu g\,ml^{-1}$) were added to the bath and left in contact with the tissue for 10 to 20 min. Then, a second cumulative concentration-response curve was obtained. Each compound was tested on separate strips and control experiments were performed with only the vehicle used to dilute these compounds (absolute ethanol), in order to correct spontaneous changes in sensitivity. When appropriate, the nominal pA_2 values as $g\,ml^{-1}$ for the different compounds against Bk- and L-Bk-induced contractions were calculated by graphic interpolation according to the method of Arunlakshana & Schild (1959). True pA_2 values could not be calculated since the molecular weights of the compounds are unknown.

Guinea-pig ileum Ileal strips about 10 to 20 mm long were obtained from guinea-pigs of either sex (300–500 g) and taken from the portion situated 10 to 30 cm proximal to the ileo-caecal junction. Preparations were set up for recording of isotonic con-

tractions in 5 ml jacketed organ baths containing Krebs Henseleit solution at 37°C continuously bubbled with air under 1 g of tension, as described previously (Calixto *et al.*, 1984). The solution had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.1, KH_2PO_4 0.9 and glucose 11. After an initial equilibration period of about 60 min, cumulative concentration-response curves for each agonist were obtained at 30 min intervals. The curves to Bk were obtained in the absence or presence of atropine (2.5 μM) or enalapril (1 μM, an inhibitor of kininase II). After obtaining two or three concentration-response curves and once the responses became reproducible, different concentrations of the compounds isolated from *M. velutina* (5 to 40 $\mu g\,ml^{-1}$) were added to the bathing solution 20 min before constructing new curves to the agonist. The mean maximal response obtained from the first two cumulative concentration-response curves was taken as the 100% response value. In separate sets of experiments, in order to correct for spontaneous and/or vehicle-induced desensitization, control experiments for Bk, oxytocin, ACh and histamine were performed in the presence of the corresponding concentrations of absolute ethanol. When appropriate, the nominal pA_2 values for Bk were calculated according the method described by Arunlakshana & Schild (1959). Only one agonist was tested in each preparation.

Statistical analysis

The results are presented, when appropriate, as mean ± s.e.mean and 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, atropine sulphate, histamine iodide (Sigma, U.S.A.), oxytocin (Syntocinon, Sandoz, Brazil), bradykinin and lysyl-bradykinin (Sigma, U.S.A.; or synthesized by the Department of Biophysics, Escola Paulista de Medicine, São Paulo, Brazil). Enalapril maleate (MK-421, $C_{22}H_{32}N_2O_4$), a dipeptide inhibitor of angiotensin converting enzyme was kindly supplied by Merck, Sharp & Dohme. Stock solutions of these drugs were prepared in water and kept at 4°C and diluted in water just before use. Oestradiol benzoate (1 $mg\,ml^{-1}$) (Sigma, U.S.A.) and compounds isolated from *M. velutina* (10 $mg\,ml^{-1}$) were dissolved in peanut oil and absolute ethanol, respectively. The final bath concentrations of ethanol were always lower than 0.01%.

Results

As shown in Figure 1 (a, b, c, d and e) the compounds isolated from *M. velutina* termed MV 8608, MV 8609, MV 8610, MV 8611, and MV 8612 (5 to 40 $\mu g\,ml^{-1}$), all caused parallel and concentration-dependent rightward displacements of the Bk (1–1000 nM) concentration-response curve. Schild plot regression lines obtained from these data (Figure 2) revealed linear relationships (correlations close to one) and gave nominal pA_2 values (as $-\log g\,ml^{-1}$) of 5.7, 5.6, 5.4, 5.7 and 5.3, respectively (Table 1). With the exception of MV 8608, the slopes of the Schild regressions did not differ significantly from unity, indicating competitive antagonism. When compared to the results obtained with the crude

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

Antagonists	pA_2^a	*Slope*[a]	*Correlation*[b]
Crude extract ($n = 7$)	3.2 (2.9–3.6)*	1.8 (1.5–2.3)	0.92 ± 0.002
MV 8608 ($n = 4$)	5.7 (5.0–6.3)	1.8 (1.2–2.4)	0.94 ± 0.03
MV 8609 ($n = 4$)	5.6 (5.1–6.1)	1.3 (1.0–1.6)	0.93 ± 0.02
MV 8610 ($n = 4$)	5.4 (5.3–5.6)	1.4 (0.8–1.8)	0.99 ± 0.007
MV 8611 ($n = 5$)	5.7 (5.2–6.1)	1.2 (0.8–1.8)	0.92 ± 0.05
MV 8612 ($n = 6$)	5.3 (5.2–5.5)	1.3 (1.0–1.6)	0.92 ± 0.04

[a] Numbers in parentheses represent 95% confidence limits ($P < 0.05$).
[b] Data shown are mean ± s.e.mean.
* Calixto *et al.*, 1985a.

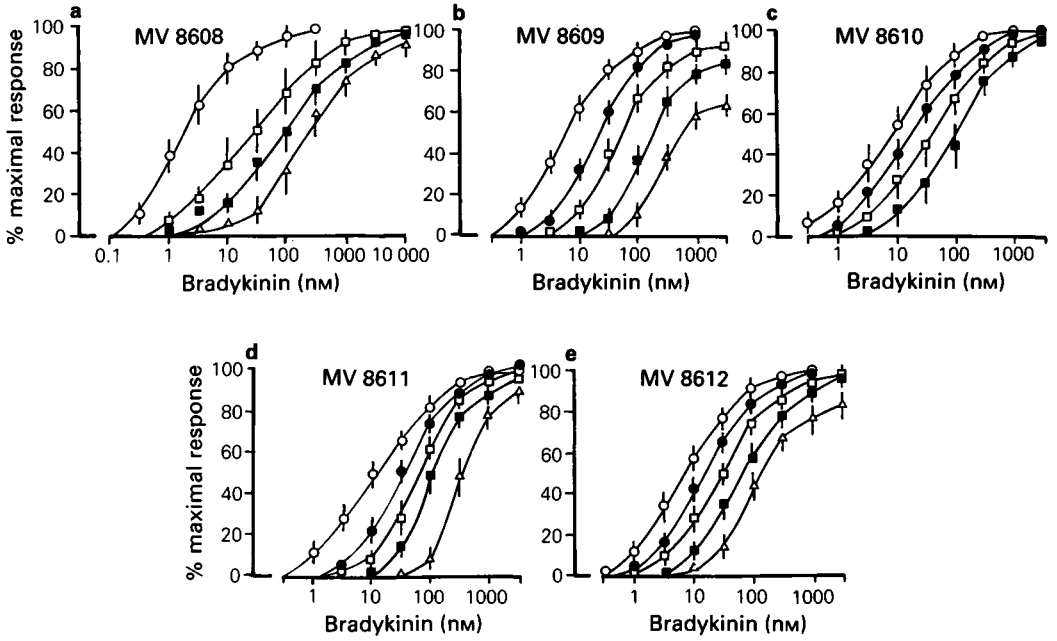

Figure 1 Mean concentration-response curves for bradykinin in the isolated uterus of the rat. Control curves (○) and responses in the presence of increasing concentration of compounds obtained from *Mandevilla velutina* ($\mu g\,ml^{-1}$): 5 (●); 10 (□); 20 (■) and 40 (△). Each point represents the mean of 4 to 6 experiments, and the vertical lines indicate the s.e.mean.

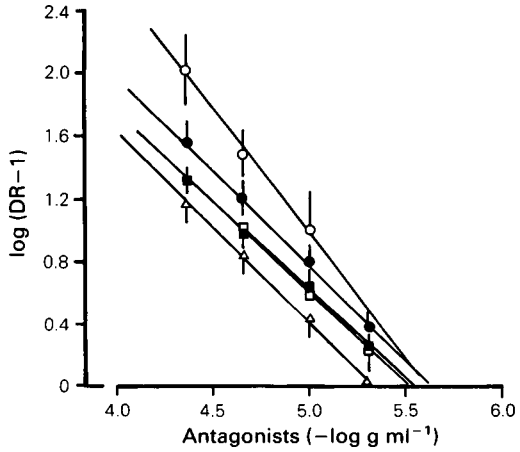

Figure 2 Schild plots for several compounds isolated from *Mandevilla velutina* as antagonists of bradykinin-induced contractions in the isolated uterus of the rat. Compounds MV 8608 (○); MV 8609 (●); MV 8610 (□); MV 8611 (■) and MV 8612 (△). The nominal pA_2 values are presented in Table 1. Each group represents the mean of 4 to 6 experiments and the vertical lines indicate the s.e.mean.

extract (Calixto *et al.*, 1985a) compounds MV 8608, MV 8609, MV 8610, MV 8611, and MV 8612 were about 316, 251, 158, 316 and 126 fold more potent, respectively, in antagonizing Bk-mediated contractions in rat uterine muscle. As previously observed in this preparation for the semipurified compounds of *M. velutina*, compounds MV 8608, MV 8611, MV 8612 (5 to 40 $\mu g\,ml^{-1}$) also caused concentration-dependent displacements to the right of the L-Bk (1 to 10,000 nM) concentration-response curve (Figure 3a, b and c). The Schild plots from these data were linear and furnished nominal pA_2 ($g\,ml^{-1}$) values of 5.4, 5.8 and 5.1, respectively, and the slopes of the regression lines did not differ from unity (Figure 4 and Table 2). These data indicate that these compounds, including the compound MV 8608, behaved as simple and competitive antagonists of L-Bk. However, the pA_2 values for these compounds against L-Bk did not differ significantly from those obtained against Bk-induced contractions. Compounds MV 8608, MV 8611 and MV 8612 were about 79, 199 and 40 fold more potent than the crude extract, respectively, in antagonizing L-Bk. Compounds MV 8609 and MV 8610 were not tested against L-Bk.

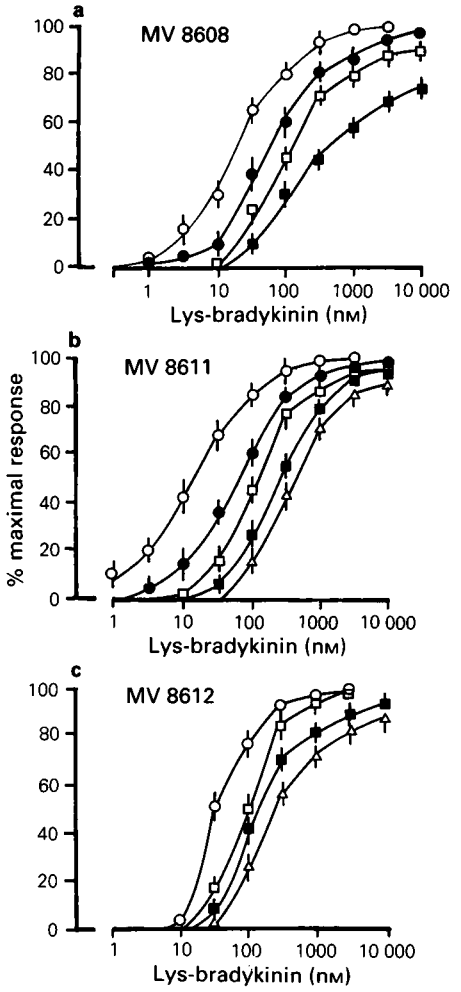

Figure 3 Mean concentration-response curves for lysyl-bradykinin in the isolated uterus of the rat. Control curves (○) and responses in the presence of increasing concentration of compounds obtained from *Mandevilla velutina* ($\mu g\,ml^{-1}$): 5 (●); 10 (□); 20 (■) and 40 (△). Each point represents the mean of 5 to 7 experiments and the vertical lines indicate the s.e.mean.

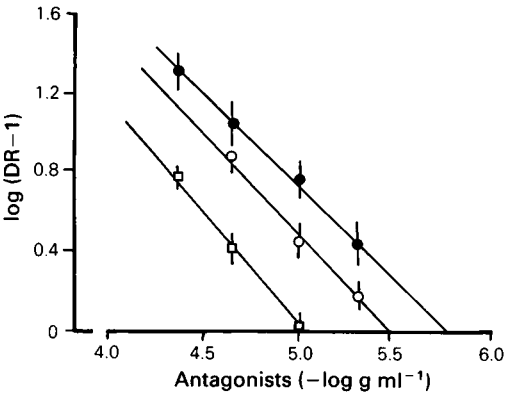

Figure 4 Schild plots for several compounds isolated from *Mandevilla velutina* as antagonist of lysyl-bradykinin-induced contractions in the isolated uterus of the rat. Compounds MV 8608 (○); MV 8611 (●) and MV 8612 (□). The nominal pA_2 values are presented in Table 2. Each group represents the mean of 5 to 7 experiments and the vertical lines indicate the s.e.mean.

When analysed against ACh-(0.1 to 100 μM) and oxytocin-(0.01 to 30 mu ml^{-1}) induced contractile responses, both MV 8608 and MV 8612 (10 and 20 $\mu g\,ml^{-1}$) failed to influence uterine contractile responses induced by these agents (dose-ratio < 2), indicating a selective action upon kinin receptors (Figure 5a, b, c and d). At concentrations greater than 40 $\mu g\,ml^{-1}$; however, the compounds decreased maximal contractions to all agonists.

In the guinea-pig ileum, compound MV 8612 (5 to 20 $\mu g\,ml^{-1}$) also caused parallel and concentration-dependent displacements to the right of the Bk (1–1000 nM) concentration-response curve, when the experiments were conducted in the presence of atropine (2.5 μM) (Figure 6c). The Schild plot of these data (Figure 6d) showed a linear relationship (correlation close to 1) and yielded a nominal pA_2 value ($g\,ml^{-1}$) of 5.2 and the slope of the regression line did not differ from unity (1.0; 95% C.L. 0.8–1.3), indicating a competitive interaction (Table 3). On the other hand, compound MV 8608 (10 to 40 $\mu g\,ml^{-1}$) also dose-dependently antagonized the Bk (1–1000 nM) concentration-response curves, but this effect was characterized by marked inhibition of the maximal responses to Bk (Figure 6a).

The different potencies exhibited by MV 8608 in antagonizing Bk-induced contractions in the rat uterus and the guinea-pig ileum could be related to the use of salt solutions with differing compositions or temperatures. To analyse these possibilities, a separate series of experiments was conducted in which we tested the effect of MV 8608 in guinea-pig ilea maintained in De Jalon solution at 37°C or in Krebs-Henseleit solution at 30°C. Reducing temperature to 30°C did not significantly modify the antagonistic effect of the compound (Figure 6b). However, in the experiments conducted in De Jalon solution at 37°C, the sensitivity of guinea-pig ileum to Bk was increased by 6 to 10 fold, whereas the antagonistic effect of MV 8608 (10 to 40 $\mu g\,ml^{-1}$) against Bk remained essentially unaltered (results not shown).

As found in the rat uterus and in the absence of atropine, compound MV 8612 (10 and 20 $\mu g\,ml^{-1}$) did not significantly affect the responsiveness of ileal

Table 2 The mean nominal pA_2 values (as $g\,ml^{-1}$) for the antagonistic effects of crude extract or compounds isolated from *Mandevilla velutina*, against lysyl-bradykinin-induced contractions in the isolated uterus of the rat

Antagonists	pA_2^a	*Slope*[a]	*Correlation*[b]
Crude extract ($n = 5$)	3.5 (3.2–3.7)*	1.7 (1.5–2.1)	0.98 ± 0.02
MV 8608 ($n = 5$)	5.4 (5.3–5.5)	1.1 (0.9–1.4)	0.95 ± 0.02
MV 8611 ($n = 5$)	5.8 (5.6–6.1)	1.0 (0.6–1.6)	0.96 ± 0.01
MV 8612 ($n = 5$)	5.1 (4.8–5.4)	0.8 (0.6–1.0)	0.92 ± 0.03

[a] Numbers in parentheses represent 95% confidence limits ($P < 0.05$).
[b] Data shown are mean ± s.e.mean.
* Calixto *et al.*, 1987a.

preparations to ACh (0.01–10 μM) or histamine (0.01–10 μM) (Figure 7c and d). However, when the compound was present at concentrations greater than $40\,\mu g\,ml^{-1}$ a significant reduction of the maximal contractile responses to both agonists was observed (results not shown). Unlike the results obtained in the rat uterus, compound MV 8608 (10 and $20\,\mu g\,ml^{-1}$) caused a slight but significant inhibition of the contractile responses of the guinea-pig ileum to ACh or histamine and decreased the sensitivity to the later agonist (Figure 7a and b). Due to the limited quantities of some of the compounds isolated from *M. velutina*, it was not possible to analyse their antagonistic effects against L-Bk in the guinea-pig

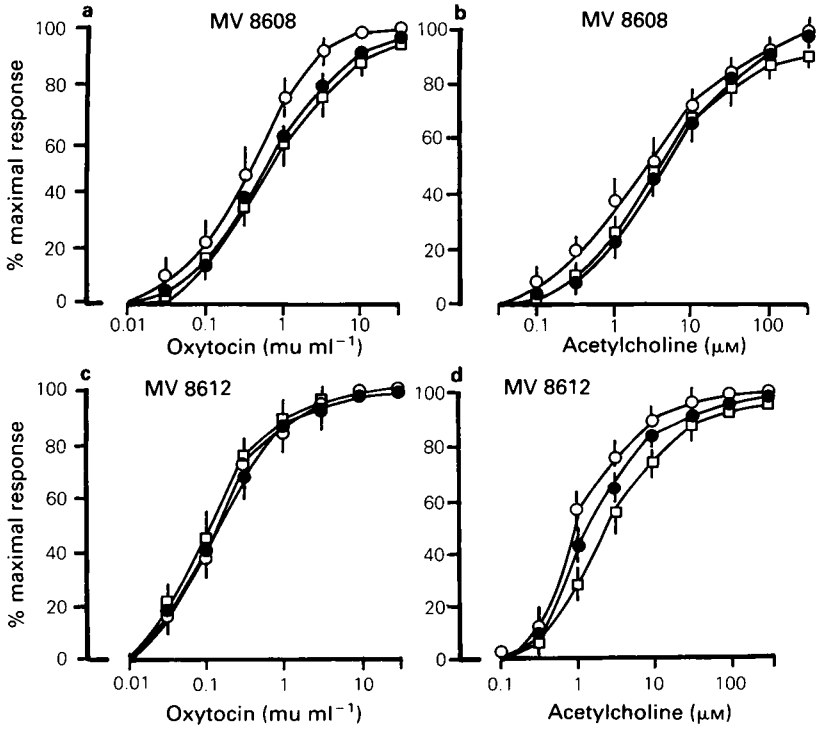

Figure 5 Mean concentration-response curves for oxytocin (a and c) and acetylcholine (b and d) in the isolated uterus of the rat. Control responses (○) and responses in the presence of compounds MV 8608 (a and b) or MV 8612 (c and d), ($\mu g\,ml^{-1}$): 10 (●) and 20 (□). Each point represents the mean of 4 to 5 experiments and the vertical lines indicate the s.e.mean.

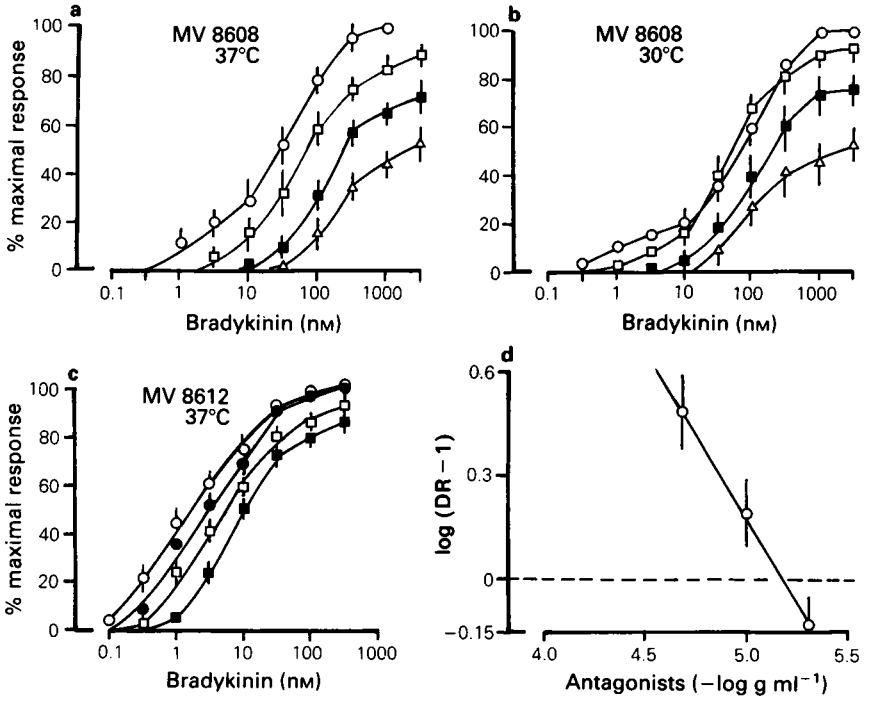

Figure 6 (a–c) Mean concentration response-curves for bradykinin in the guinea-pig isolated ileum. Control curves (○) and responses in the presence of increasing concentration of compounds obtained from *Mandevilla velutina* ($\mu g\,ml^{-1}$): 5 (●); 10 (□); 20 (■) and 40 (△). (d) Schild plot for compound MV 8612 as antagonist of bradykinin-induced contractions in the guinea-pig isolated ileum. The nominal pA_2 value is presented in Table 3. Each point represents the mean of 5 to 6 experiments and the vertical lines indicate the s.e.mean.

ileum or their selectivities against other agonists in both preparations.

None of the compounds tested showed agonistic activity in either the rat uterus or the guinea-pig ileum. The onset of anti-Bk activity of these compounds isolated from *M. velutina* was rapid (within 10 to 20 min) and was reversible following intermittent washing of both preparations for 30–60 min (results not shown).

Discussion

The present findings provide additional evidence for the existence of several non-peptide compounds in

Table 3 The mean nominal pA_2 values (as $g\,ml^{-1}$) for the antagonistic effects of crude extract and compounds isolated from *Mandevilla velutina*, against bradykinin-induced contractions in the guinea-pig isolated ileum

Antagonists	*pA_2*[a]	*Slope*[b]	*Correlation*
Crude extract ($n = 6$)	3.2 (2.9–3.6)*	1.5 (1.1–1.9)[a]	0.98 ± 0.01
MV 8608 ($n = 5$)	5.3 (5.1–5.5)	c	—
MV 8612 ($n = 5$)	5.2 (4.8–5.6)	1.0 (0.8–1.3)	0.90 ± 0.06

[a] Numbers in parentheses represent 95% confidence limits ($P < 0.05$).
[b] Data shown are mean ± s.e.mean.
[c] Since compound MV 8608 decreased the maximal response induced by bradykinin, the slope could not be calculated.
* Calixto *et al.*, 1987b.

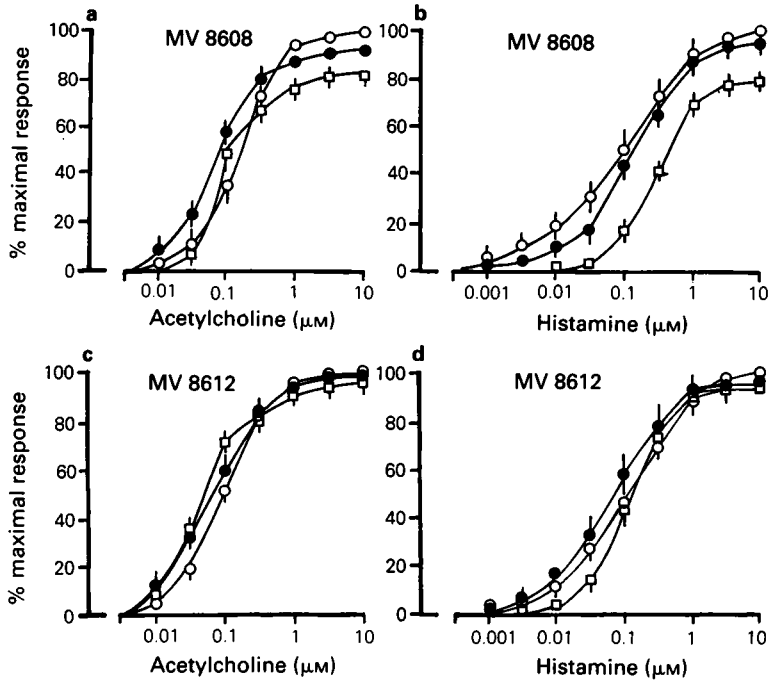

Figure 7 Mean concentration-response curves for acetylcholine (a and c) and histamine (b and d) in the guinea-pig isolated ileum. Control response (○) and response in the presence of compound MV 8608 (a and b) and MV 8612 (c and d) obtained from *Mandevilla velutina* ($\mu g\,ml^{-1}$): 10 (●) and 20 (□). Each point represents the mean of 4 to 5 experiments and the vertical lines indicate the s.e.mean.

the crude extract of *M. velutina* which can selectively and competitively antagonize the contractile responses to Bk and L-Bk, thus corroborating previous studies (Calixto *et al.*, 1985a,b; 1987a,b; Calixto & Yunes 1986). Of the five compounds tested against Bk in the rat uterus, only MV 8608 did not fulfil Schild's criteria for a simple competitive mechanism of anti-Bk action, since the slopes of the Schild plots for the other four compounds were not different from 1 (Tallarida *et al.*, 1979; Kenakin, 1982; 1984). Also in line with our previous data, obtained with fractions isolated from *M. velutina* (Calixto *et al.*, 1987a), the antagonistic effects of the purified compounds were clearly concentration-dependent and reversible. Moreover, at concentrations lower than $40\,\mu g\,ml^{-1}$, MV 8608 and MV 8612 failed to affect the responsiveness of the rat uterus to ACh and oxytocin, suggesting that the antagonistic action of at least these two compounds is selective against Bk-related peptides.

Both MV 8608 and MV 8612 also antagonized Bk-induced contractions in the guinea-pig ileum. The antagonism exerted by the former compound was characterized by concentration-dependent rightward shifts of the curve to Bk combined with significant and proportional reductions of the maximal response. In contrast, compound MV 8612 clearly antagonized Bk-induced contractions in a simple competitive fashion. Also, unlike MV 8608, compound MV 8612 did not affect the responsiveness of the guinea-pig ileum to ACh or histamine at concentrations below $40\,\mu g\,ml^{-1}$. It should be mentioned, however, that when the concentration of these compounds was increased to $40\,\mu g\,ml^{-1}$ or more, significant depressions of the maximal responses to Bk and other agonists were observed in both preparations. Therefore, the selectivity of MV 8608 and MV 8612 in antagonizing Bk-induced responses only occurs within a limited concentration range. Unfortunately, the limited amounts of MV 8609, MV 8610 and MV 8611 precluded an analysis of their anti-Bk effects in the guinea-pig ileum and of their selectivity in either preparation.

Uterine contractions induced by Bk and L-Bk were similarly affected by compounds MV 8608, MV 8611 and MV 8612. The inability of the compounds to distinguish between the kinins reinforces our previous suggestion, based on studies using crude or semi-purified extracts of *M. velutina* (Calixto *et al.*, 1985a; 1987a), that Bk and L-Bk interact with a

common site in this preparation. In the guinea-pig ileum, the apparent pA_2 value for compound MV 8612 against Bk was not different from that obtained in the rat uterus, which would suggest the presence of similar kinin receptors in both preparations. However, when the results obtained with compound MV 8608 against Bk are compared, a clear difference in the profiles of antagonism between the preparations becomes evident. Although this compound displaced Bk curves to the right in both cases, only in the guinea-pig ileum was this effect accompanied by depression of the maximal response. This differential action of MV 8608 in the rat uterus and guinea-pig ileum cannot be ascribed to differences in the temperatures at which antagonism was tested or in the compositions of the nutrient solutions and, thus, could reflect the existence of distinct kinin receptors in the two tissues.

The antagonistic effect of these compounds against Bk was only observed in the guinea-pig ileum when the experiments were conducted in the presence of atropine. This condition was also found to be important for the expression of the anti-Bk activity of the crude extract of *M. velutina* (Calixto *et al.*, 1987b). Recently, it has been reported that contractions of the guinea-pig ileum induced by Bk involve not only a direct action on the smooth muscle cells, but also the release of prostaglandin E_2, which in turn stimulates the release of ACh (Goldstein *et al.*, 1983; Yau *et al.*, 1986). It is also well known that kinins can promote a biphasic effect in isolated intestine, i.e., relaxation followed by contraction, which depends on the existence of previous tonus (Antônio, 1968; Hall & Bonta, 1973; Boschov *et al.*, 1984). Such findings could reflect the existence of different kinin receptors in this tissue. Indeed, two distinct binding sites for Bk and related peptides have been detected in the guinea-pig ileum (Manning *et al.*, 1986). Kinin receptor heterogeneity in the guinea-pig ileum has also been proposed by Innis *et al.* (1981), based on correlations between the rank orders of potency of kinin related peptides in competing for Bk-binding sites and in promoting contraction. These authors also provided evidence for the occurrence of a single type of kinin receptor in the rat myometrium, a view that has received increasing support (Odya *et al.*, 1980; Yasujima *et al.*, 1982; Frederick *et al.*, 1984; Frederick & Odya, 1987). Taken together, these studies strongly indicate that kinin receptors can vary between tissues. However, considerable variation between species can also occur, as demonstrated recently by Plevin & Poat (1987) in relation to the renal cortical kinin receptors of the rat and the guinea-pig. A possible interaction with kinin-receptors situated in the ileal mucosa (Cuthbert & Margolis, 1982; Manning *et al.*, 1982; Hojvat *et al.*, 1983; Cox *et al.*, 1986; Hoult & Phillips, 1986) cannot be discarded. Recently, it has been shown that the Bk-receptors mediating ileal secretion and contraction are similar and of the B_2-type (Kachur *et al.*, 1987).

In summary, these results confirm and extend our previous studies, indicating the existence of several non-peptide compounds in the roots of *M. velutina* which can act as simple, competitive and selective antagonists at kinin receptors in the rat uterus and in the guinea-pig ileum. We are currently investigating whether these compounds are also responsible for the effects induced *in vivo* by the crude extract of *M. velutina*. Moreover, molecular characterization of some of these compounds strongly suggests that their anti-Bk activity occurs within the micromolar range.

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