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NATURAL BRADYKININ ANTAGONISTS

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Bradykinin (BK) a nonapeptide generated in plasma during tissue injury, is involved in many physiological and pathological states. Kinin actions are mediated by specific membrane receptors and involve a complex signal transducer and also second messager mechanisms. Due to its inequivocal relevance, an intensive effort has been focused in recent years to develop selective and competitive BK antagonists. Thus, the development of a new series of peptide BK antagonists has made an important contribution to the understanding of the pharmacological, physiological and pathophysiological role of BK, and this is certain to provide a firm basis for developing new drugs to relieve pain and inflammation. However, BK antagonists derived from peptide origin reported to date have limited clinical use due to their poor oral absorbtion and short duration of effect. Thus, considerable effort has also been made in developing stable nonpeptide BK antagonists. Up to now, most nonpeptide compounds reported to exhibit BK antagonistic activity have been derived from plants, including many flavonoids, terpenes, and also synthetic substances with various molecular structures. Amongst them, the pregnane glycoside compounds isolated from the plant Mandevilla velutina *are the most promising. These compounds are effective in antagonizing BK responses in a variety of preparations, and they also exhibit potent and long-lasting analgesic and anti-inflammatory activities. The exact mechanism underlying their action however, is not yet completely understood.*

Key words: Bradykinin antagonists – *Mandevilla velutina* – analgesic – inflammation

Bradykinin (BK, NH -Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-COOH) and the decapeptide kallidin (N-Lysyl-BK) are produced from high molecular weight kininogen precursor by the action of protease, known as a soluble or glandular kallikrein in response to tissue injury or infection. Since the pioneer study of Rocha e Silva et al. (1949), there is a considerable amount of evidence indicating that kinins are involved in a wide variety of physiological and pathological processes. Accordingly, they cause vasodilation, increase vascular permeability, stimulate and sensitize the nociceptive neurons producing pain and can enhance secretory functions of the gastrointestinal mucosa. In addition, kinins are implicated in the ethiology of several inflammatory diseases, including arthritis, allergic rhinitis, asthma, anaphylaxis, pancreatites and inflammatory bowel diseases (for review see: Rocha e Silva, 1963; Kelermeyer & Graham 1968a, b; Colman & Wong 1979; Regoli & Barabe, 1980; Proud & Kaplan, 1989; Burch et al., 1990).

In spite of several decades of investigation and the systematic studies on the structure-activity relationship of many BK analogues, until recently no competitive and selective BK antagonists have been available (Regoli & Barabe, 1980). The absence of such antagonists has caused difficulty over a long period in the understanding of the importance of kinins in many physiological and pathological conditions. More recently, a series of BK analogues has been described which includes specific and competitive antagonists (for review see: Steranka et al., 1988, 1989; Burch et al., 1990). By the use of such antagonists it was possible to characterize multiple BK receptors named B_1 and B_2. Recent studies suggest that all the most important physiological BK actions are presumably mediated by the B_2 receptor type and that multiple B_2 receptors may exist (Regole & Barabe, 1980; Steranka et al., 1988; 1989; Burch et al., 1990).

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In the first part of this short review we describe the anti-BK action of some naturally occurring substances. The second part discusses some recent *in vitro* and *in vivo* data on kinin antagonistic activity for the extract and natural compounds from the plant *Mandevilla velutina.*

NONPEPTIDE BRADYKININ ANTAGONISTS FROM NATURAL PRODUCTS

Several compounds from natural origin have been studied for potential BK antagonist activity. The flavonoids apiin and hesperitin and the related bronchodilator and antispasmodic furanochromone khellin were found effective in antagonizing BK-induced contraction of the isolated guinea-pig ileum, but only khelin behaved as a competitive antagonist (Garcia Leme & Walaszeck, 1973). However, their antagonistic activity was not selective, since all three compounds affected responses to angiotensin II and eledoisin. In addition, khelin and apiin given systemically, inhibited BK-and-carrageenan-induced rat paw oedema (Garcia Leme & Walaszeck, 1973). Chau & Halley (1969), also reported that other flavonoids, including quercetin, rhamnetin, homoeriodictyol and, to a lesser extent, morin and sculin, have been shown to antagonize BK response in guinea-pig ileum. Similar results were obtained for the biflavonoids amentoflavone from *Ginko biloba* and cupressuflavone from *Cupressus tarubosa* (Ramaswamy, 1970). Another flavonoid, o-(β-hydroxyethyl)-rutin also exhibits anti-BK activity in the rat uterus at high concentrations (Garbor, 1979). Vitexin, a flavone glycoside from *Ochrocarpus longitofilius* L and *Arneba hispidissimica* Dc, also antagonize BK response in the guinea-pig ileum and present anti-inflammatory activity (Prabshakar et al., 1981). However, the funtional anti-BK properties of all these substances must be analyzed with caution, because data regarding their selectivity and mechanism of action are still lacking.

Recently, we have found that acetylated glycosides eucryphin and astilbin, from *Hymenaea martiana* and the crude extract from the bark of this plant (Leguminosae) antagonize in a concentration-dependent manner, BK-induced contraction of the isolated rat uterus, but these compounds and the extract are not selective to kinins, since they inhibit responses to other agonists (Calixto et al., 1989). The diterpene jotrophone, isolated from *Jatropha elliptica* (Euphorbiaceae), also antagonizes in a concentration-dependent and non-competitive manner, BK-induced contractions of the isolated rat uterus and guinea-pig ileum (Calixto & Sant'Ana, 1987). The saponin from horse chestnut aescin exhibits functional antagonism against BK-induced increase of vascular permeability and presents a lymphopenic action (Vogel, 1971).

Interestingly, vitamin K_3 (mentadione) and streptomycin antagonize in a concentration-dependent and noncompetitive fashion, BK-induced contraction of the guinea-pig ileum (Altinkurt & Kanzik 1980a). However, as observed before, these compounds are not selective to kinins, since responses to acetylcholine and histamine were also affected. Both substances also blocked the bronchoconstriction caused *in vivo* by BK in guinea-pigs, but enhanced responses to histamine (Altinkurt & Kanzik, 1980 b).

Depending on the tissue and enzyme present, binding of BK at B_2 receptors can lead to activation of several second messager transducer mechanisms. Thus, the non-selective anti-BK profile of the mentioned natural compounds may include inhibition of eicosanoid synthesis via reduction of cyclo and lypoxigenase and/or phospholipase A_2 activities, blockade of phospholipase(s) C mediated phosphathidylnositol pathways, inositoltriphosphate and diacylglycerol accumulation, protein quinase C activation or calcium mobilization. In fact, quercetin and kaempferol are capable of interfering with protein quinase C and myosin light chain kinase, respectively (Picp et al., 1989; Rogers & Willians 1989). Additionally, the protein quinase inhibitor, the alkaloid staurosporine, isolated from *Streptomyces* sp., inhibits BK-and the diterpene phorbol ester-induced activation of peripheral capsaicin-sensitive nociceptors. Both effects are reversibly antagonized by the B_2 BK-receptor antagonists, indicating that B_2 receptors linked with nociceptors are coupled to protein kinase C (Dray & Perkins, 1988).

NONPEPTIDE BRADYKININ ANTAGONISTS FROM *MANDEVILLA VELUTINA*

Mandevilla velutina (Apocynaceae) is a native plant to Brazil, and its rhizomes are used in folk medicine to treat poisonous snake bites; they are also used as an anti-inflammatory

preparations. The previous evidence that *Bothrops jararaca* venoms can release (Rocha e Silva et al., 1949) and potentiate BK responses (Ferreira, 1965) prompted us to investigate whether extracts of rhizome of this plant display anti-BK activity.

Effect on isolated smooth muscles – The crude extract (CE) of *M. velutina* functionally antagonized BK-induced uterine contraction. This effect was of rapid onset, reversible and selective, since responses to acetylcholine, oxytocin and angiotensin II were unaffected (Calixto et al., 1985 a). Subsequently, we demonstrated that the CE also antagonizes response to lysyl-BK of the rat uterus and contraction of the guinea-pig ileum and urinary bladder caused by BK, L-BK and methionyl-L-BK (Calixto et al., 1988 b; Calixto & Yunes, 1990). Although Schild analysis revealed similar pA_2 values for the CE in antagonizing BK responses, the slopes of Schild regression were significantly higher than unity, indicating possible existence of several anti-BK active compounds in the CE (see Table). The CE was found to antagonize contractions mediated by both B_1 (des-Arg^9)-BK and B_2 BK receptor in vascular beds from rabbits (Calixto & Yunes, 1986). The actions appear to be selective to BK, since the contractile response to noradrenaline was virtually unaffected. The CE also antagonized BK-induced potentiation of electrically-evoked nerve-mediated responses in the isolated guinea-pig bladder (Calixto & Yunes, 1990). In addition, the contractile response evoked by BK of the isolated cat ileum was also antagonized by *M. velutina* CE, leaving response to acetylcholine and histamine unaffected (Calixto et al., 1988 b). Similar selective BK-antagonistic activity was also detected in the rat stomach preparation (M. Nicolau, unpublished results).

Two semipurified fractions obtained from the CE (fraction 11 and 12) were about 80 to 160 fold more potent in antagonizing BK-induced uterine contractile response than the CE (Calixto et al., 1987). In addition, their antagonistic actions were selective in nature furnishing Schild regression not different from unity. Further purification of these fractions led to the obtaining of five non-peptides which functionally antagonize BK-induced contraction in the rat uterus (Calixto et al., 1988a). Chemical analysis revealed that four of these compounds are steroidal glycosides (MV 8609, MV 9610, MV 8611 and MV 9612, with molecular weights ranging from 527 to 1182) and the fifth (MV 8608) was the aglycone steroid (MW 362; R. A. Yunes & J. B. Calixto, manuscript submitted for publication). Although all five compounds functionally antagonize BK response in the rat uterus, only MV 8612 behaves as a selective BK antagonist in the guinea-pig ileum (Table). The other four compounds present essentially similar pA_2 values to those of MV 8612, (ranging between 2 and 14 μM), but they also caused an inhibition of BK maximal response (Table).

The CE and compound MV 8608 and MV 8612 antagonize both the contractile and relaxant response caused by kinin of the isolated rat duodenum (Calixto et al., 1985 b; 1988 c). Again, the antagonistic effects are quite selective, since the relaxant response to adrenaline and the diphasic response to KCl were only marginally affected. Additionally, the CE was found to reverse BK-induced endothelium-dependent relaxation in dog femoral and mesenteric artery rings precontracted by noradrenaline (Calixto et al., 1985 b). This action appears to be quite selective against BK, since the vasodilation caused by acetylcholine was only partially affected (about 20% of the preparation). However, the CE failed to antagonize BK relaxation in rabbit aortic rings, but markedly inhibited dilatation triggered by acetylcholine and calcium ionophoro A 23187. Also, the CE markedly antagonizes histamine-mediated relaxation in rat aortic rings (J. B. Calixto, unpublished results). Neither the CE nor the pure compounds from *M. velutina* affected responses to endothelium-independent vasodilators such as papaverine and sodium nitroprusside.

More recently, we have investigated the anti-BK action of the CE and pure compounds obtained from *Mandevilla illustris*, another plant belonging to the same genus as *M. velutina*, also used as a folk medicine against snake bites. The CE and purified prenane compounds from of this plant also dose-dependently antagonized kinin-induced contractile effect of the rat uterus. However, this functional action was nonselective, since this CE also affected uterine contraction to other oxytocic agents (Calixto & Yunes, 1991; Calixto et al., 1991 a). In spite of presenting the same potency as *M. velutina* compounds, these compounds lack selectivity against BK (Calixto et al., 1991 b).

TABLE

The mean pA_2 values for the antagonistic effects of the crude extract and several compounds from *Mandevilla velutina* against kinin-induced contractions in isolated smooth muscle preparations

Antagonist	Agonist	Rat uterus		Guinea-pig ileum		Guinea-pig Urinary bladder	
		pA_2[a]	Slope	pA_2[a]	Slope	pA_2[a]	Slope
CE	BK	3.2	1.8[d]	3.2	1.5[d]	3.5	2.2[d]
	L-BK	3.5	1.7[d]	3.8	1.1	3.7	2.4[d]
	ACh	no effect		–		no effect	
	OT	no effect		–		–	
	His	–		–		no effect	
	AII	no effect		–		–	
MV8608	BK	5.3	1.8[d]	4.9	*b*	not tested	
	L-BK	5.0	1.1	–			
	ACh	no effect		no effect			
	OT	no effect		–			
	His	–		*c*			
MV8609	BK	5.3	1.3	5.2	*b*	not tested	
	L-BK	–		–			
	ACh	no effect		no effect			
	OT	no effect		–			
	His	no effect		*c*			
MV8610	BK	5.3	1.4	5.2	*b*	not tested	
	ACh	no effect		no effect			
	OT	no effect		–			
	His	–		*c*			
MV8611	BK	5.3	1.2	5.3	*b*	not tested	
	L-BK	5.8	1.0				
	ACh	no effect		no effect			
	OT	no effect		–			
	His	–		*c*			
MV8612	BK	5.4	1.3	5.2	1.0	not tested	
	L-BK	5.2	0.8	–			
	ACh	no effect		no effect			
	OT	no effect		–			
	His	–		no effect			

Each group represents the mean of 4 to 8 experiments.
a: pA_2 values (-log of antagonist concentration which caused a 2 fold displacement to the right of the agonist concentration-response curves) are in molar basis, except for the CE (as g/ml).
b: the slope could not be calculated, since maximal response was inhibited.
c: compound at 20 μg/ml affected histamine response.
d: significantly different from unity ($p < 0.05$).
Bradykinin (BK), Lysyl-bradykinin (L-BK), acethylcholine (ACh), oxytocin (OT), histamine (His), angiotensin II (AII).
Results from Calixto et al., 1985 a; 1988 a, b; Calixto & Yunes, 1990.

A non-active alcohol pregnane compound named illustrol has also been recently isolated from this plant rhizome (Yunes et al., 1989).

Anti-inflammatory and anti-nociceptive properties – The CE of *M. velutina* given orally (50-200 mg/kg) exhibited a potent and long-lasting anti-oedematogenic activity against several phlogistic agents, but was more effective against BK and cellulose sulphate-induced paw oedema (ED_{50} of 70 and 86 mg/kg, respectively). The CE also inhibited to a lesser extent, paw oedema caused by carrageenan, PAF-acether, zymosan, serotonin and dextran (Calixto et al., 1986; Henriques et al., 1991; Calixto et al., 1991 b). Interestingly, the CE even at

higher doses (400 mg/kg), failed to interfere with *B. jararaca*-induced paw oedema. We have recently demonstrated that this oedema is mediated mainly by cyclo-oxygenase products of arachidonic acid metabolism, activation of both alfa$_1$ and alfa$_2$ adrenoceptors, whereas histamine, serotonin and PAF contribute only discretely (Trebien & Calixto, 1989). In contrast, kinins appear to be not involved in *B. jararaca* paw oedema.

Compound MV 8610 (60-240 nmol/paw) and to a greater extent, MV 8612 (40-170 nmol/paw), dose-dependently antagonized BK (3 nmol/paw)-induced rat paw oedema. Similar anti-oedematogenic action was observed when both compounds (5-10 μmo/kg) were injected intraperitoneally (Neves et al., 1989).

The CE of *M. velutina* also exhibited anti-inflammatory activity triggered by BK, cellulose sulphate, PAF, carrageenan and serotonin in mouse paw oedema (Henriques et al., 1989). Compound MV 8612 (40-170 nmol/paw), injected intradermically, exhibited marked anti-inflammatory profile against BK (50 nmol/paw) and carrageenan (100 μg/paw)-induced mouse paw oedema (ED_{50} of 52 and 48 nmol/paw, respectively) (Neves et al., 1989). Compound MV 8608 was less active and MV 8610 had no effect. Interestingly, at the same dose compound MV 8612 failed to affect PAF and serotonin paw oedema (Neves et al., 1989).

The CE (50-400 mg/kg PO), was found effective in antagonizing arachidonic acid (AA)-induced ear oedema in mice (ED_{50} of 70 mg/kg) (Calixto et al., 1991 c). Compound MV 8612 applied topically (96-500 nmol/ear), or given by IP route, (2.5-25 μmol/kg), caused a clear and dose-dependent inhibition of AA ear oedema (ED_{50S} of 163 nmol/ear and 6.2 μmol/kg, respectively). Compound MV 8610 was less potent, whereas MV 8608 caused only a mild inhibition. However, both MV 8608 and MV 8612 failed to affect prostaglandin synthesis of rat stomach preparations (Calixto et al., 1991 c).

The CE of *M. velutina* (50-200 mg/kg, PO) markedly antagonized pleural exudate acumulation caused by BK and cellulose sulphate. Response to PAF, serotonin, zymosan and carrageenan were less affected (Henriques et al., 1991 a). The CE also inhibited to a lesser extent pleural exudation caused by BK, carrageenan, serotonin and PAF in mice (Henriques et al., 1989). However, the CE failed to affect differential leukocite counts in both species.

The increases of vascular permeability caused by BK and *B. jararaca* venom in rats have been found to be antagonized by IP injection of the CE, without affecting responses to histamine (Nicolau et al., 1986). By contrast, intradermic (160-560 nmol/site) or intravenous (0.8-3 μmol/kg) injection of compounds MV 8610 and MV 8612 or MV 8608, all fail to antagonize BK and *B. jararaca* venom enhanced of vascular permeability (Neves et al., 1989).

The inhibitory action of the CE of *M. velutina* against PAF-acether-induced paw oedema and pleurisy does not seem related to the blockade of PAF receptors. Both compounds MV 8612 and MV 8610, at concentrations 100-fold greater than those necessary to functionally antagonize kinin response *in vitro*, failed to affect PAF-acether and thrombin-induced platelet aggregation in humans (Coelho et al., 1989). At very high concentration MV 8608 antagonized the aggregatory action of PAF and ADP ($ED_{50} \cong 1$ mM).

In the same doses that these compounds exhibited anti-inflammatory profile, compound MV 8612 (0.25-8.4 μmol/kg, ip) was found to inhibit in a dose-dependent fashion nociceptive response caused by acetic acid in mice (ED_{50} of 1.2 μmol/kg). Compound MV 8610 was slightly less potent, whereas MV 8608 caused only partial inhibition of writhes (J. B. Calixto, unpublished results).

Effect on ovalbumin-induced anaphylaxis – Pretreatment of ovalbumin sensitized guinea-pigs with the CE of *M. velutina* (60 mg/kg, iv) 15 min before antigen challenge, significantly reduced the hypotension and mortality caused by ovalbumin, without interfering with the bronchoconstrictor response. Both compounds MV 8608 (3 μmol/kg, iv) and MV 8612 (0.8 μmol/kg iv) reduced the mortality and hypotension induced by ovalbumin, and caused a mild but significant inhibition or bronchoconstriction (Bauman et al., 1989; Calixto et al., 1991 b). Response to other agonists, like acetylcholie, histamine and serotonin were not affected by the CE of *M. velutina*. In addition, compound MV 8612, but not MV 8608, significantly antagonized BK-induced hypotension in guinea pigs.

In sensitized guinea-pigs pretreated with pyrilamine plus indomethacin, compound MV 8612 (0.8-5 μmol/kg iv) dose-dependently antagonized albumin-induced bronchoconstriction. Compound MV 8610 (up to 7 μmol/kg) and MV 8608 (up to 16 μmol/kg) had no effect (Bauman et al., 1989). These findings strongly suggest that the CE and particularly MV 8612 attenuate anaphylaxis in ovalbumin sensitized guinea-pig, mainly by interfering with the cardiovascular component of the reaction. These actions appear to be unrelated with histamine release, since both compounds failed to inhibit histamine release from rat peritoneal mast cells (Bauman et al., 1989).

CONCLUSIONS

In the effort to develop new BK antagonists, a large number of nonpeptide natural substances have been screened. These compounds include most flavonoids and some terpenoids, which are effective in antagonizing BK actions in different *in vitro* and *in vivo* models, although with limited selectivity. Since BK action involves several signal transducer mechanisms it is possible that such substances may interfere with one or more steps of these intracellular mechanisms.

Concerning the *M. velutina* compounds, the acummulated findings strongly suggest that such steroid substances may constitute a new class of putative nonpeptide BK antagonists. However, binding studies showing that such compounds can displace radiolabelled kinin from their receptors must still be provided in order to consider them true competitive BK antagonists. Thus, possible interference of these compounds with intracellular signal transducer mechanisms triggered by BK action cannot yet be excluded. Despite this fact, these compounds may constitute a potentially novel class of analgesic and anti-inflammatory drugs.

Finally, since rhizomes from *M. velutina* furnish low yields of active compounds, and in view of the difficulty of obtaining large amounts of this plant, we have recently obtained some active anti-BK compounds from the plant through callus culture *in vitro* (Calixto et al., 1989 b; Silva et al., 1989 a), as well as mass *in vitro* propagation of *M. velutina* (Silva et al., 1989 b). Both approaches constitute rapid and efficient means for large scale production of these active principles.

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