Current Trends Review

Bradykinin-Antagonists: Therapeutic Perspectives

J.E. Taylor, F.V. DeFeudis, and J.P. Moreau

Biomeasure Inc., Hopkinton (J.E.T., J.P.M.) and Institute for BioScience, Inc., Grafton (F.V.D.), Massachusetts

INTRODUCTION—ACTIONS OF BRADYKININ
BRADYKININ RECEPTORS
MECHANISMS OF ACTION OF BRADYKININ
ROLES OF ENDOTHELIUM-DERIVED RELAXING FACTORS
THERAPEUTIC GOALS: BRADYKININ ANTAGONISTS
COMPETITIVE BRADYKININ ANTAGONISTS
ANTINOCICEPTIVE EFFECTS OF BRADYKININ ANTAGONISTS
BRADYKININ ANTAGONISTS AND CIRCULATORY RESPONSES
BRADYKININ ANTAGONISTS AND INFLAMMATION
MULTIPLE RECEPTORS FOR BRADYKININ: HETEROGENEITY OF B_2 RECEPTORS
WHAT SHOULD BE EXPECTED OF A NEW BRADYKININ ANTAGONIST?
CONCLUDING COMMENTS

ABSTRACT

Taylor, J.E., F.V. DeFeudis, and J.P. Moreau: Bradykinin-antagonists: Therapeutic perspectives. Drug Dev. Res. **16**:1–11, 1989.

In vitro and in vivo studies of the actions of various antagonists and agonists of bradykinin (BK) B_1 and B_2 receptors have been reviewed and analyzed. It seems apparent that certain B_2-antagonists, such as [Thi^{5,8},D-Phe^7]-BK and D-Arg^0-[Hyp^3,Thi^{5,8},D-Phe^7]-BK will serve as valuable model substances for developing new anti-inflammatory/analgesic drugs. Further development of B_1-antagonists, based on modifications of the structure of des-

Received final version July 19, 1988; accepted August 4, 1988.

Address reprint requests to J.E. Taylor, Biomeasure, Inc., 11-15 E Avenue, Hopkinton, MA 01748.

Arg^9,[Leu^8]-BK, or combined B_1/B_2-antagonists might be useful in controlling disease states involving chronic tissue injury and/or prolonged vasospasm.

Key words: bradykinin (BK), vasodilatation, BK antagonists, pain, inflammation, vascular endothelial cells, prostacyclin (PGI_2), endothelium-derived relaxing factor(s) (EDRF), des-Arg^9-BK, des-Arg^9,[Leu^8]-BK, [Thi^{5,8},D-Phe^7]-BK, D-Arg^0-[Hyp^3, Thi^{5,8},D-Phe^7]-BK, multiple BK receptors, therapeutic applications

INTRODUCTION—ACTIONS OF BRADYKININ

The nonapeptide bradykinin (BK; Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) is one of the principal mediators that is released when the body responds to trauma and injury. Like other kinins, BK influences vascular tone and permeability, decreases blood pressure, and initiates or enhances the release of mediators from leukocytes [see Parker, 1984; Sharma, 1988]. Such properties indicate that BK is involved in pain, inflammation, and in certain allergic reactions. BK is produced from large molecular weight precursors (kininogens) via the actions of kallikreins (which generally appear to be Arg-esterases). This occurs in plasma or tissue during the activation of clotting and proteolysis [Coleman, 1974; Regoli and Barabé, 1980]. BK is normally present in very low concentration in plasma and tissue due to its rapid degradation (plasma half-life <30 sec) [see Parker, 1984]. It is catabolyzed either by removal of Arg via the action of Kininase-I (carboxypeptidase N) or by removal of Phe-Arg via the action of Kininase-II (angiotensin-converting enzyme) from its C-terminal [Regoli and Barabé, 1980]. Des-Arg^9-BK formed via Kininase-I action possesses greater or less activity than BK itself, depending on the assay system employed; des-Phe^8,Arg^9-BK formed via Kininase-II action, appears to be inactive.

The cascade of biochemical reactions initiated by tissue injury and leading to the synthesis of BK (and other kinins), in common with coagulation and fibrinolysis, is caused by activation of Hageman factor [see Parker, 1984; Proud and Kaplan, 1988]. Administration of Kininase-II inhibitors, like captopril, can enhance some actions (e.g., the edema-inducing activity) of BK [see Clark et al., 1988]. Locally injected BK causes pain, swelling, and other signs of inflammation. Such actions appear to be related to BK's actions of stimulating the synthesis and/or release of prostacyclin (PGI_2) or "endothelium-derived relaxing factor(s)" (EDRF) in vascular endothelial cells (see below).

BRADYKININ RECEPTORS

BK acts as a local hormone in mediating cellular and vascular actions [see Rocha e Silva, 1970; Regoli and Barabé, 1980]. Its effects can involve different sites, i.e., vascular endothelial cells, smooth muscle fibers, autonomic nerve endings of resistance vessels, afferent nociceptive nerve endings, or the CNS [see Regoli, 1984]. Many factors, for example, species, blood vessel examined, and experimental conditions (in vitro vs. in vivo) can influence the actions that follow activation of BK receptors. At least two distinct receptor types, B_1 and B_2, appear to exist [Regoli and Barabé, 1980; Vavrek and Stewart, 1985; Bouthillier et al., 1987]. Although activation of B_2 receptors appears to underlie the most relevant biological actions of kinins (e.g., pain, inflammation), both B_1 and B_2 receptors could be important in developing therapeutic strategies, and therefore, both will be discussed herein.

Activation of B_1 receptors, which exhibit the special feature of being generated de novo in isolated tissue preparations (e.g., rabbit isolated aorta) or in response to pathological states in vivo, generally causes stimulation of smooth muscle cells of various tissues, increased cell proliferation, and increased collagen synthesis [Regoli, 1984]. B_1 receptors mediate contraction of arterial and venous preparations in vitro, but can also cause relaxation of peripheral

TABLE 1. Biological Activities of Bradykinin and Related Peptides on Isolated Vascular and Smooth Muscle Preparations*

	Rabbit aortic strip			Rabbit jugular vein			Dog carotid artery		
Compound	α^E	pD_2	R^A	α^E	pD_2	R^A	α^E	pD_2	R^A
Bradykinin (BK)	1.0	6.22	8.5	1.0	8.46	100.0	1.0	8.64	100.0
Lys-BK (KD)	1.0	7.27	95.0	1.0	8.63	150.0	1.0	8.44	64.0
Des-Arg^9-BK	1.0	7.29	100.0	—	4.39	0.01	—	—	<0.01
Des-Arg^{10}-KD	1.0	8.61	2100.0	—	5.33	0.06	1.0	7.22	3.8
[Tyr(Me)^8]-BK	1.0	4.70	<0.01	1.0	8.59	140.0	1.0	8.64	100.0
		pA_2			pA_2			pA_2	
Des-Arg^9,[Leu^8]-BK		7.27			Inactive			Inactive	
[Pro^4,Trp^{7,9,10}]-SP		Inactive			5.20			5.70	
[Pro^4,Trp^{7,9},pNO$_2$Phe^8]-SP (4–11)		Inactive			5.43			Inactive	

*α^E, intrinsic activity; pD_2, apparent affinity expressed by the $-\log$ of the concentration that produces 50% on the maximum effect; RA, relative affinity in percent of that of des-Arg^9-BK in the rabbit aorta, and of bradykinin in the other two preparations; pA_2, $-\log$ of the concentration of antagonist that reduces the effect of a double to that of a single dose of agonist; KD, kallidin. (TABLE 1 reproduced with permission from Journal of Cardiovascular Pharmacology, Volume 6; Regoli, D.: Neurohumoral regulation of precapillary vessels: The Kallikrein-Kinin system. Copyright 1984, Raven Press.)

resistance vessels in vivo (see Regoli, [1984]). It is of interest that the number of B_1 receptors increases during tissue injury and that des-Arg^9-BK, formed via the action of Kininase-I (see above), is more potent than BK itself in stimulating these receptors [Regoli and Barabé, 1980]. Thus, even though B_1 antagonists are not active on the biological actions of BK that are mediated by B_2 receptors, the induction of B_1 receptors that occurs during tissue injury, coupled with degradation of BK to des-Arg^9-BK, indicates that the development of B_1-antagonists might be fruitful.

Activation of B_2 receptors generally mediates pain and vasodilatation, enhances the release of histamine and prostaglandins (PGs), stimulates smooth muscle cells of various tissues, and activates sensory nerves and reflexes [Regoli, 1984]. Thus, B_2 receptors mediate relaxation of arteries, contraction of veins, and BK-induced increases in vascular permeability (i.e., extravasation, which commences at venular sites and appears to involve endothelial B_2 receptors) [Regoli, 1984; Unterberg and Baethmann, 1984].

Vasorelaxant effects of BK that occur via stimulation of B_2 receptors in isolated large arteries of some species (e.g., dog, pig, man) require intact vascular endothelial cells and, therefore, depend upon endothelial B_2 receptors [Regoli, 1984; Beny et al., 1987] (see below). Vasocontractile effects of BK, regardless of whether these are mediated by activation of B_1 or B_2 receptors, do not require an intact endothelium and appear to depend upon the B_1 or B_2 receptors that are present on smooth muscle cells [Regoli, 1984; Beny et al., 1987]. BK-induced hypotension is generally mediated by B_2 receptors, but B_1 receptors can be involved under certain conditions (see Regoli, [1984]).

Pharmacological data concerning the activities of BK and some related peptides on three isolated vascular preparations are provided in Table 1 [Regoli, 1984]. It is noteworthy that in rabbit aorta the most potent agonists lack the C-terminal Arg residue, whereas in rabbit jugular vein and dog carotid artery the most potent agonists are BK itself and [Tyr(Me)^8]-BK; i.e., the order of potency of BK-agonists in rabbit aorta (which has B_1-receptors) is opposite to those of rabbit jugular vein and dog carotid artery (both of which possess B_2-receptors). Also, des-Arg^9[Leu^8]-BK, a potent antagonist on rabbit aortic strip, is inactive in the other two preparations, and [Pro^2,Trp^{7,9,10}]-substance P (SP) is inactive on rabbit aortic strip but active

on the two other preparations (Table 1). In this regard, the B_1 antagonist des-Arg^9-BK is much less active than BK itself on rat uterus and guinea pig ileum (which contain mainly B_2 receptors), and the reverse holds for rabbit aorta [see Parker, 1984].

Receptors for BK exist in many tissues (e.g., nervous system, epithelia, smooth muscle cells, fibroblasts); and in each tissue BK can elicit specific responses, including muscle contraction, fluid secretion (by epithelia), neurotransmitter release, and stimulation of cellular growth. BK might even act as a neurotransmitter in certain cases, and it can activate sensory afferent fibers that transmit nociceptive information to the CNS and the release of SP in the dorsal horn [see Miller, 1987]. High-affinity binding sites for BK exist in all tissues that are sensitive to BK (see below).

MECHANISMS OF ACTION OF BRADYKININ

Interaction of BK with membrane receptors stimulates the production of various active lipid-derived intermediates, including inositol triphosphate, diacylglycerol, arachidonate, and the various cyclooxygenase (CO) and lipoxygenase products of arachidonate (see Miller [1987]; Proud and Kaplan [1988]). These substances can increase cellular concentrations of "second messengers" such as cyclic-AMP, cyclic-GMP, and Ca^{2+}, which, in turn, can activate certain enzymes (e.g., various kinases). Release of such substances would involve the activation of phospholipase-C and/or phospholipase-A_2, and in certain cases some type of G_i/G_o-like G protein could also be involved in the transduction mechanism [see Miller, 1987].

ROLES OF ENDOTHELIUM-DERIVED RELAXING FACTORS

Vascular endothelial cells play significant roles in various pathophysiological responses, including inflammation, wound-healing, and atherogenesis, and BK has pronounced actions on these cells, at least in vitro. In relaxing isolated arteries, BK acts by one of two different *indirect* mechanisms, depending on the species and blood vessel examined (see Furchgott [1984]). De-endothelialization of superior mesenteric and celiac arteries of rabbit and cat does not usually decrease their sensitivities to the vasorelaxant effect of BK, and in these preparations BK-induced relaxation is blocked by CO-inhibitors (e.g., indomethacin) indicating that it is mediated by PGs (see Furchgott [1984]). In contrast, canine arteries require intact endothelial cells for BK-induced relaxation, and these responses are not blocked by CO-inhibitors, indicating that they depend upon EDRF (see Furchgott [1984]). Threshold concentrations for this latter action of BK were 0.1–1.0 nM. In human mesenteric arteries and ovarian artery and in pig aorta BK also causes relaxation by endothelium-dependent, PG-independent mechanisms [Gordon and Martin, 1983; Furchgott, 1984]. In sum, BK-induced vasorelaxation appears to be mediated by PGs (probably mainly PGI_2) in arteries of cats and rabbits, but by EDRF (possibly nitric oxide, at least in some blood vessels) [Palmer et al., 1987] in arteries of dog and human.

With regard to the development of therapeutic agents, it is of interest that BK-induced relaxation appears to be mediated by EDRF in human arteries. Could such actions occur in resistance vessels in vivo? Since BK might be formed in, or released directly into, the bloodstream, the answer would be positive. This type of mechanism could occur in humans in vivo [see Furchgott, 1984]. Most recently, Ignarro et al. [1987] have shown that BK relaxes bovine intrapulmonary artery by endothelium-dependent mechanisms involving the actions of both cyclic GMP and cyclic AMP, whose formation might be stimulated by EDRF and PGI_2, respectively, but that it relaxes intrapulmonary vein by endothelium-dependent mechanisms involving only cyclic GMP. Such results offer a model system for examining the effects of BK analogs on EDRF- versus PGI_2-mediated BK-ergic mechanisms.

THERAPEUTIC GOALS: BRADYKININ ANTAGONISTS

The apparent physiological actions of BK in mediating pain and inflammation and in regulating blood pressure have been mentioned above. If certain BK antagonists are as effective in human skin as in rabbit skin and if they block BK-mediated pain in man, these could be useful anti-inflammatory and/or analgesic agents [Schachter et al., 1987] (see below). Rationale supporting the use of BK antagonists as analgesics is strengthened by the findings that BK is present in rat spinal cord [Perry and Snyder, 1984] and in cerebrospinal fluid [Thomas et al., 1984]. Since BK release and hyperalgesia are components of superficial pain associated with burns and inflammation, topically applied BK antagonists could be effective analgesics [Steranka et al., 1987] (see below).

The possible involvement of kallikrein-kinin systems in the brain's cerebrovascular responses to other pathophysiological phenomena, such as concussive brain injury or acute hypertension which injure blood vessels by a CO-dependent mechanism, might also involve BK [Ellis et al., 1987]. Also, BK causes cerebral edema, which is associated with increased brain concentrations of BK [Unterberg and Baethmann, 1984; Maier-Hauff et al., 1984; Wahl, 1985]. BK antagonists could be useful in controlling these conditions.

In general, the major therapeutic goals would include testing various B_1 and B_2 antagonists for their effects on pathological conditions caused by either enhanced production or inadequate destruction of BK and related kinins. In addition to their possible use in controlling superficial inflammation and pain, BK antagonists might be of some value in treating rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, allergic rhinitis, asthma, and gout [Proud and Kaplan, 1988]. Therapeutic rationale is strengthened upon considering B_1 and B_2 receptors (i.e., formation of des-Arg^9-BK in a given tissue could terminate biologic activity if B_2 receptors exist or enhance activity if B_1 receptors predominate) [see Proud and Kaplan, 1988]. This provides flexibility for approaches aimed at developing BK analogs as therapeutic agents. Further flexibility derives from the recent finding that certain BK analogs appear to interact with a multiplicity of B_2-receptor subtypes [Braas et al., 1988] (see below).

COMPETITIVE BRADYKININ ANTAGONISTS

For developing antagonists to BK receptors, B_1- and B_2-mediated actions of BK must be considered. Although B_2 receptors appear to mediate the most characteristic actions of BK, B_1 receptors that can be induced during pathological states in vivo might be important in relation to certain disease states and tissue injury, especially since des-Arg^9-BK and des-Arg^{10}-kallidin (which activate B_1 receptors) resist degradation in the lung [Marceau et al., 1983].

B_1 antagonists, identified by Regoli and Barabé [1980], are selective, specific, rapidly reversible, and competitive. These are octapeptide analogues of des-Arg^9-BK and have affinities similar to those of the most potent natural agonists [Regoli, 1984, 1985] (see Table 1). Extension of the peptide chain at the N-terminal by adding a Lys residue increases the affinity of these B_1 antagonists by more than one log unit while restoring some B_2 agonist activity [Regoli, 1985]. Des-Arg^9,[Leu^8]-BK is a prototype competitive B_1 antagonist; its pA_2 value is 7.27 on rabbit aorta [Regoli, 1985].

Sequence-related B_2-receptor antagonists have been more recently identified [Vavrek and Stewart, 1985]. Replacement of the Pro^7 residue of BK with a D-Phe residue is the major modification which converts BK agonists to BK antagonists. [D-Phe^7]-BK exhibits moderate ($pA_2 \simeq 5.0$) inhibition of BK activity on guinea pig ileum but possesses weak BK-like myotropic activity on the isolated rat uterus and 2–4% of BK depressor potency in the rat blood pressure assay. Replacement of the Phe residues at positions 5 and 8 of [D-Phe^7]-BK with isosteric β-(2-thienyl)-alanine residues (Thi) produced a potent antagonist of BK. This substance, [Thi^{5,8},D-Phe^7]-BK is active on uterus ($pA_2 \simeq 6.4$), ileum ($pA_2 \simeq 6.3$), dog

carotid artery ($pA_2 \simeq 6.4$), and in the rat blood pressure assay [Vavrek and Stewart, 1985; Regoli, 1985]. This antagonism of BK's actions on smooth muscle was fully reversible and specific for kinins (BK, kallidin, Met-Lys-BK); smooth muscle activity of angiotensin-II or SP were not antagonized. BK analogs containing D-Phe residues are likely to resist enzymatic degradation since they are not hydrolyzed by Kininase-II of small intestine and lung.

Newer synthetic B_2 antagonists have been synthesized and tested on rabbit blood vessels and guinea pig ileum [Schachter et al., 1987]. One of these, D-Arg°-[Hyp^3,Thi^{5,8},D-Phe^7]-BK, effectively antagonized BK-induced vascular permeability in rabbit skin. Its selectivity for B_2 receptors was confirmed by showing that the selective B_1 antagonist des-Arg^9-[Leu^8]-BK did not antagonize the increase in vascular permeability produced by intradermally injected BK.

ANTINOCICEPTIVE EFFECTS OF BRADYKININ ANTAGONISTS

Many studies have indicated that BK serves as a physiological mediator of hyperalgesia (e.g., see above). In this regard, Steranka et al. [1987] have examined the antinociceptive effects of two BK antagonists that were previously shown to be potent inhibitors of [^{3}H]-BK binding in guinea pig ileum. Intraperitoneal injection of both substances, D-Arg°-[Hyp^3,Thi^{5,8},D-Phe^7]-BK (termed NPC-349) and D-Arg°[Hyp^3,D-Phe^7]-BK (termed NPC-567), blocked acetic-acid-induced writhing in mice in dose-dependent fashion, NPC-349 being slightly more potent than NPC-567. Both substances, injected intradermally at doses tenfold greater than that of BK, also blocked BK-induced hyperalgesia, tested by examining the ability of BK to accentuate the nociceptive effects of applied paw pressure in the rat. Griesbacher and Lembeck [1987], using the reflex hypotensive response produced by injection of BK into the ear artery of anesthetized rabbits as an indicator of nociception, showed that this response can be antagonized by a local infusion of Lys-Lys-[Hyp^3,Thi^{5,8},D-Phe^7]-BK (50 and 500 nM). This antagonism was dose-dependent, reversible, and apparently competitive.

These results have been confirmed and extended in recent studies which have shown that [^{3}H]-BK binding sites can be localized autoradiographically to the substantia gelatinosa, dorsal horn, and dorsal root of spinal cord and to myocardial/coronary visceral afferent fibers in the guinea pig [Manning and Snyder, 1983; Steranka et al., 1988], areas which contain receptors to nociceptive pathways. Furthermore, certain BK antagonists, developed as B_2 antagonists, such as Lys-Lys-[Hyp^{2,3},Thi^{5,8},D-Phe^7]-BK or [Leu^{5,8},Gly^6,D-Phe^7]-BK, blocked BK-induced acute vascular pain in the rat and relieved BK- and urate-induced hyperalgesia in the rat paw. Certain BK antagonists blocked urate-induced hyperalgesia even when administered after the development of altered pain sensitivity [Steranka et al., 1988]. These results strongly support the contention that BK is a physiological mediator of pain and indicate further that B_2 antagonists have analgesic activity in both acute and chronic pain models. Most of the analogs tested showed potent activity in both vascular and hyperalgesia models, but [D-Nal^1,Thi^{5,8},D-Phe^7]-BK did not block BK-induced vascular pain or ileal contractions while antagonizing BK-induced rat paw hyperalgesia and BK-induced contraction of the rat uterus. These interesting findings indicate that the B_2 receptor mediating *vascular pain* might differ from that involved in *cutaneous hyperalgesia*. This element of specificity should be important in designing tissue-selective BK antagonists.

The BK receptor localizations found by Steranka et al. [1988] could be related to the pain transmission associated with angina. In this regard, they recalled studies which showed that coronary artery constriction causes the release and accumulation of BK in the coronary vasculature and myocardium, and that BK might cause coronary pain by stimulating cardiovascular visceral afferent nociceptors. However, activation of B_2-type receptors (localized to endothelial cells) by BK would be expected to cause relaxation rather than constriction (see above). Des-Arg^9-BK, in contrast, causes contraction (e.g., of pig coronary artery) which

does not depend upon the endothelium and which is mediated directly via activation of the B_1 receptors of arterial smooth muscle [Beny et al., 1987]. The repressed B_1 receptors of pig isolated coronary arteries have been shown to become expressed during the artificial conditions of in vitro incubation. On this basis, Beny et al. [1987] have suggested that certain pathological states, like in vitro conditions, would favor the synthesis of B_1 receptors and that this could be significant since des-Arg^9-BK would then induce prolonged coronary artery contraction (e.g., a mimic of coronary vasospasm which is implicated in angina pectoris in man). This analysis indicates that B_1 antagonism as well as B_2 antagonism might be necessary for controlling the two major components of angina, coronary vasospasm and pain, and that development of combined B_1/B_2 antagonists of BK might be fruitful. Tissue selectivity, rather than receptor (B_1 vs. B_2) specificity might be the most important therapeutic goal in relation to controlling angina.

BRADYKININ ANTAGONISTS AND CIRCULATORY RESPONSES

Using the dog hind-limb preparation for bioassay, Beierwaltes et al. [1987] found that three BK analogs produced significant parallel shifts in the femoral vasodilatatory response to BK. D-Arg^0-[Hyp^3,Thi^{5,8},D-Phe^7]-BK, the most potent analog tested, produced a full log dose shift in the dose-response curve to BK at the 4-nmol/min infusion rate. None of these analogs elicited vasodilatation themselves or affected systemic blood pressure at the doses tested.

Close-arterial injection of D-Arg^0-[Hyp^3,Thi^{5,8},D-Phe^7]-BK (50 nmol) into the cat submandibular gland reduced the effect of a close-arterial injection of BK (0.1 nmol) to about 9% of the control value after 1 min and to about 42% and 71% after 15 and 30 min, respectively [Barton et al., 1987]. This B_2 antagonist (250 nmol, i.v.) also reduced or abolished the hypotensive action of BK (1–5 nmol, i.v.) in the cat and rabbit without affecting the hypotensive action of acetylcholine [Barton et al., 1987]. Continuous infusion of this same substance into the carotid artery of binephrectomized rats completely blocked the vasodilatatory response of BK in submandibular gland when BK was given in a bolus dose 2.5–10 times higher than that required for maximal vasodilatation [Berg et al., 1987]. Also, [Thi^{5,8},D-Phe^7]-BK (6 μM) inhibited BK-induced cerebral arteriolar dilatation, measured as rabbit pial arteriole diameter using the closed cranial window technique [Ellis et al., 1987]. Cerebral arteriolar dilatation produced by adenosine, acetylcholine or vasoactive intestinal polypeptide was not altered by this latter agent, revealing its specificity for BK receptors.

Effects of B_2 antagonists on BK-induced plasma extravasation, venoconstriction, and PGE_2 release have also been reported [see, e.g., Griesbacher and Lembeck, 1987]. Taken together, these studies, which have shown the abilities of various B_2 antagonists to inhibit circulatory actions of BK, have far-reaching possibilities both for examining the pathophysiological roles of BK and for establishing therapeutic agents that will reverse certain BK-induced effects.

BRADYKININ ANTAGONISTS AND INFLAMMATION

BK and other kinins act on resistance vessels as well as on capillaries and veins in a fashion resembling that of other pro-inflammatory agents. By reducing the tone of small arteries, BK elicits vasodilatation, thereby increasing blood flow which contributes to inflammation [Regoli, 1984]. Vasodilatation is caused by endothelium-dependent relaxation of arterial smooth muscle by BK, whereas BK-induced increases in vascular permeability appear to involve constriction of venules and reduction of the length of capillary endothelial cells. Hence, by decreasing arteriolar resistance, BK elicits increases in pressure and fluid of the

capillary bed, thereby favoring efflux of fluid from blood to tissues (extravasation). Increases in capillary permeability and in venous pressure caused by BK further enhance this efflux [Regoli and Barabé, 1980; Marceau et al., 1983; Regoli, 1984]. These effects of BK (i.e., its pro-inflammatory actions) appear to involve primarily the activation of B_2 receptors. B_2 antagonists which have been shown to inhibit BK's actions of eliciting vasodilatation and enhancing plasma extravasation have antiinflammatory activity in various animal models (see above).

New analgesic/antiinflammatory agents are needed due to the many problems associated with the use of non steroidal antiinflammatory drugs [e.g., Simon and Mills, 1980a,b] and other antiinflammatory agents (e.g., corticosteroids), but certain problems must be overcome in developing useful B_2 antagonists. Beneficial actions of BK could be inhibited by blockade of B_2 receptors, leading to undesirable side effects such as Na^+ retention, arterial constriction, increased plasma volume, or hypertension. With regard to hypertension, endogenous BK might normally play a significant role in cardiovascular regulation via its actions of inhibiting the pressor effects of angiotensin-II, vasopressin, and α-adrenergic stimulation [Aubert et al., 1988].

MULTIPLE RECEPTORS FOR BRADYKININ: HETEROGENEITY OF B_2 RECEPTORS

The existence of multiple receptor subtypes for BK is evident from the B_1- versus B_2-receptor classification (see above). Further subdivision of the B_2 class is also possible upon considering the various tissue-selective effects of BK and BK-analogs. This is an important concept since the various effects elicited by BK (e.g., its nociceptive, vasodilatatory, and inflammatory actions) might be subserved by different types of B_2 receptors. Some evidence supports the idea that the B_2-receptor-mediating vascular pain might differ from that subserving cutaneous hyperalgesia [Steranka et al., 1988]. Such differences might permit the eventual design of BK antagonists with greater selectivity for a given tissue receptor of the B_2 type.

The study of Rifo et al. [1987] which showed a heterogeneity of BK-induced responses in rat vas deferens is also of interest. Application of BK to the isolated transmurally stimulated vas deferens of rat caused two effects: an increase in the basal tension of the tissue (postjunctional effect) and a potentiation of the magnitude of electrically driven twitches (prejunctional effect). Tyr-BK, [Tyr^5]-BK, and [Tyr^8]-BK showed significant differences in their potency ratios in producing these responses. [$Thi^{5,8}$,D-Phe^7]-BK acted as a full agonist in potentiating electrically induced twitches (neuronally mediated BK response), but antagonized BK-induced postjunctional contraction. D-Arg^0-[Hyp^3,$Thi^{5,8}$,D-Phe^7]-BK had no agonist activity at either pre- or postjunctional sites, but behaved as a potent pure antagonist. Addition of the D-Arg residue at the N-terminal and hydroxylation of Pro at the 3 position increased this antagonist's potency. These B_2 antagonists were specific in that they did not modify significantly the magnitude of the contactile responses caused by angiotensin-II, norepinephrine, or serotonin. This discrimination between the receptor involved in the two BK-mediated responses of vas deferens provides further support for a heterogeneity of B_2 receptors.

Earlier studies conducted with [^{3}H]-BK had shown that two types of binding site might exist on uterus membranes [Oyda et al., 1980] and on neuroblastoma clone N1E-115 cells [Snider and Richelson, 1984]. Also, Manning et al. [1986] detected two binding processes for [^{3}H]-BK in guinea pig heart, kidney, and ileum smooth muscle. This binding was not modified by des-Arg^9-BK, indicating the presence of two types of B_2 receptors. Most recently, Braas et al. [1988], upon examining the effects of BK antagonists on [^{3}H]-BK binding and inositol phosphate production in neuroblastoma N1E-115 cells, also concluded that multiple B_2-receptor subtypes might exist.

WHAT SHOULD BE EXPECTED OF A NEW BRADYKININ ANTAGONIST?

The design of a new BK antagonist would depend upon the type of pathological process considered. Actions at specific sites involved in the mediation of inflammatory, nociceptive, and/or vasodilatatory processes would require BK antagonists selective to B_2 receptors or to a given B_2 receptor subtype. The findings that B_2 receptors are heterogeneous and that BK elicits endothelium-dependent vasorelaxation, which could involve either PGI_2 or EDRF as mediator, provides flexibility for such an approach. Development of a B_2 antagonist that would selectively inhibit either the PGI_2- or EDRF-mediated endothelium-dependent vasodilation elicited by BK would represent a major advance with respect to tissue selectivity and specificity of action. Actions at specific sites involved in coronary vasospasm or chronic tissue injury might require BK antagonists selective to those B_1 receptors that can be activated by des-Arg^9-BK. If one considers therapy for pathological states that involve both the inflammatory pain and the chronic tissue injury that are caused by BK and des-Arg^9-BK, respectively, then combined (nonselective) BK antagonists might be the most useful agents for therapy.

Regardless of the type of antagonist developed, resistance to the actions of Kininase-I and Kininase-II is also of major concern. However, this problem has already been largely overcome by substituting non-natural amino acid residues into the chemical structure of BK.

With regard to route of administration of BK antagonists, topical [Ellis et al., 1987], intracutaneous [e.g., Griesbacher and Lembeck, 1987], intravenous [Griesbacher and Lembeck, 1987], and intra-arterial [Barton et al., 1987] routes have been used with some success. If one considers the possible use of B_2 antagonists for treating noninfectious inflammation and superficial pain, the topical route would be sufficient. If, however, one considers inflammatory and hyperalgesic mechanisms related to "deep" tissues, also involving vasodilatatory actions of BK, then perhaps it would be necessary to administer B_2 antagonists by other routes. Therapy for angina might require administration of B_1 or B_1/B_2 antagonists via intravenous or intra-arterial routes.

CONCLUDING COMMENTS

It seems apparent, from the studies reviewed herein, that the development of BK antagonists represents an important area for continued research. Antagonists of B_2 receptors should be useful in controlling the inflammation, pain, and vasodilatation that characterize a wide variety of pathological states. B_1 antagonists or combined B_1/B_2 antagonists might be found useful in treating other disease processes involving chronic tissue injury or prolonged vasospasm that might be mediated by the des-Arg^9-BK formed via the action of Kininase-I. Care should be taken to ensure that vital actions of BK are not inhibited to any marked extent by administration of BK antagonists. Further study of the mechanisms of action of BK in eliciting various responses, especially at the level of the specific mediators involved in its vasodilatatory effects in various circulatory beds, should provide greater insight for designing more selective BK antagonists. BK antagonists that are more potent, and in some cases more selective, are required for modulating the many important pathophysiological actions of BK.

REFERENCES

Aubert, J.F., Waeber, B., Nussberger, J., Vavrek, R., Stewart, J.M., and Brunner, H.R.: Influence of endogenous bradykinin on acute blood pressure response to vasopressors in normotensive rats assessed with a bradykinin antagonist. J. Cardiovasc. Pharmacol. **11**:51–55, 1988.

Barton, S., Karpinski, E., and Schachter, M.: Synthetic competitive antagonists of bradykinin on vasodilator responses in the cat and rabbit. J. Physiol. (Lond.) **391**:68P, 1987.

Beierwaltes, W.H., Carretero, O.A., Scicli, A.G., Vavrek, R.J., and Stewart, M.J.: Competitive analog antagonists of bradykinin in the canine hindlimb. Proc. Soc. Exp. Biol. Med. **186:**79–83, 1987.

Beny, J.L., Brunet, P., and Huggel, H.: Interaction of bradykinin and des-Arg^9-bradykinin with isolated pig coronary arteries: Mechanical and electrophysiological events. Regul. Pept. **17:**181–190, 1987.

Berg, T., Carretero, O.A., and Stewart, J.: The effect of bradykinin antagonist in rat submandibular gland blood flow regulation. J. Physiol. (Lond.) **390:**110P, 1987.

Bouthillier, J., Deblois, D., and Marceau, F.: Studies on the induction of pharmacological responses to des-Arg^9-bradykinin in vitro and in vivo. Br. J. Pharmacol. **92:**257–264, 1987.

Braas, K.M., Manning, D.C., Perry, D.C., and Snyder, S.H.: Bradykinin analogues: Differential agonist and antagonist activities suggesting multiple receptors. Br. J. Pharmacol. **94:**3–5, 1988.

Clark, E.J., Brain, S.D., and Williams, T.J.: The importance of kininase-II in the inactivation of bradykinin in the rabbit. Br. J. Pharmacol. [Suppl.] **93:**81P, 1988.

Coleman, R.W.: Formation of human plasma kinin. N. Engl. J. Med. **291:**509–515, 1974.

Ellis, E.F., Heizer, M.L., Hambrecht, G.S., Holt, S.A., Stewart, J.M., and Vavrek, R.J.: Inhibition of bradykinin- and kallikrein-induced cerebral arteriolar dilation by a specific bradykinin antagonist. Stroke **18:**792–795, 1987.

Furchgott, R.F.: The role of endothelium in the responses of vascular smooth muscle to drugs. Annu. Rev. Pharmacol. Toxicol. **24:**175–197, 1984.

Gordon, J.L. and Martin, W.: Endothelium-dependent relaxation of the pig aorta: Relationship to the stimulation of Rb efflux from isolated endothelial cells. Br. J. Pharmacol. **79:**531–541, 1983.

Griesbacher, T. and Lembeck, F.: Effect of bradykinin antagonists on bradykinin-induced plasma extravasation, venoconstriction, prostaglandin E_2 release, nociceptor stimulation and contraction of the iris sphincter muscle in the rabbit. Br. J. Pharmacol. **92:**333–340, 1987.

Ignarro, L.J., Byrns, R.E., Buga, G.M., and Wood, K.S.: Mechanisms of endothelium-dependent vascular smooth muscle relaxation elicited by bradykinin and VIP. Am. J. Physiol. **253:**H1074–H1082, 1987.

Maier-Hauff, K., Baethmann, J., Lange, M., Schurer, L., and Unterberg, A.: The Kallikrein kinin system as mediator in vasogenic brain edema. Part 2. Studies on kinin formation in local and perifocal brain tissue. J. Neurosurg. **61:**97–106, 1984.

Manning, D.C. and Snyder, S.H.: [^{3}H] Bradykinin receptor localization in spinal cord and sensory ganglia: Evidence for a role in primary afferent function. Neurosci. Abstr. **9:**590, 1983.

Manning, D.C., Vavrek, R., Stewart, J.M., and Snyder, S.H.: Two bradykinin binding sites with picomolar affinities. J. Pharmacol. Exp. Ther. **237:**504–512, 1986.

Marceau, F., Lussier, A., Regoli, D., and Giroud, J.P.: Pharmacology of kinins: Their relevance to tissue injury and inflammation. Gen. Pharmacol. **14:**209–229, 1983.

Miller, R.J.: Bradykinin highlights the role of phospholipid metabolism in the control of nerve excitability. Trends Neurosci. **10:**226–228, 1987.

Odya, C.E., Goodfriend, T.L., and Pena, C.: Bradykinin receptor-like binding studies with iodinated analogs. Biochem. Pharmacol. **29:**175–185, 1980.

Palmer, R.M.J., Ferrige, A.G., and Moncada, S.: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature **327:**524–526, 1987.

Parker, C.W.: Mediators: release and function. In Paul, W.E. (ed): "Fundamental Immunology." New York: Raven Press, 1984, 697–747.

Perry, D.C. and Snyder, S.H.: Identification of bradykinin in mammalian brain. J. Neurochem. **43:**1072–1080, 1984.

Proud, D. and Kaplan, A.P.: Kinin formation: Mechanisms and role in inflammatory disorders. Annu. Rev. Immunol. **6:**49–83, 1988.

Regoli, D.: Neurohumoral regulation of precapillary vessels: The kallikrein-kinin system. J. Cardiovasc. Pharmacol. **6:**S401–S412, 1984.

Regoli, D.: Peptide antagonists. Trends Pharmacol. Sci. **6:**481–484, 1985.

Regoli, D. and Barabé, J.: Pharmacology of bradykinin and related kinins. Pharmacol. Rev. **32:**1–46, 1980.

Rifo, J., Pourrat, M., Vavrek, R.J., Stewart, J.M., and Huidobro-Toro, J.P.: Bradykinin receptor antagonists used to characterize the heterogeneity of bradykinin-induced responses in rat vas deferens. Eur. J. Pharmacol. **142:**305–312, 1987.

Rocha e Silva, M.: "Kinin Hormones." Springfield, IL: Charles C. Thomas, 1970.

Schachter, M., Uchida, Y., Longridge, D.J., Labedz, T., Whalley, E.T., Vavrek, R.J., and Stewart, J.M.: New synthetic antagonists of bradykinin. Br. J. Pharmacol. **92:**851–855, 1987.

Sharma, J.N.: Interrelationship between the kallikrein-kinin system and hypertension: A review. Gen. Pharmacol. **19:**177–187, 1988.

Simon, L.S. and Mills, J.A.: Nonsteroidal anti-inflammatory drugs. N. Engl. J. Med. **302:**1179–1185, 1980a.

Simon, L.S. and Mills, J.A.: Nonsteroidal anti-inflammatory drugs. N. Engl. J. Med. **302:**1237–1243, 1980b.

Snider, R.M. and Richelson, E.: Bradykinin receptor-mediated cyclic GMP formation in a nerve cell population (murine neuroblastoma clone N1E-115). J. Neurochem. **43:**1749–1754, 1984.

Steranka, L.R., DeHaas, C.J., Vavrek, R.J., Stewart, J.M., Enna, S.J., and Snyder, S.H.: Antinociceptive effects of bradykinin antagonists. Eur. J. Pharmacol. **136:**261–262, 1987.

Steranka, L.R., Manning, D.C., DeHaas, C.J., Ferkany, J.W., Borosky, S.A., Connor, J.R., Vavrek, R.J., Stewart, J.M., and Snyder, S.H.: Bradykinin as a pain mediator: Receptors are localized to sensory neurons, and antagonists have analgesic actions. Proc. Natl. Acad. Sci. U.S.A. **85:**3245–3249, 1988.

Thomas, G.R., Thibodeaux, H., Margolius, H.S., and Privatera, P.J.: Cerebrospinal fluid kinins and cardiovascular function, effects of cerebroventricular Melittin. Hypertension **6**(Suppl. 1):I-46, 1984.

Unterberg, A. and Baethmann, A.J.: The kallikrein-kinin systems as mediators in vasogenic brain edema. Part 1. Cerebral exposure to bradykinin and plasma. J. Neurosurg. **61:**87–96, 1984.

Vavrek, R.J. and Stewart, J.M.: Competitive antagonists of bradykinin. Peptides (Fayetteville) **6:**161–164, 1985.

Wahl, M.: Local chemical, neural, and humoral regulation of cerebrovascular resistance vessels. J. Cardiovasc. Pharmacol. **7**(Suppl. 3):36–46, 1985.