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Competitive Analog Antagonists of Bradykinin in the Canine Hindlimb[1] (42588)

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Abstract. Six structural analogs of bradykinin were tested to determine whether they antagonize the vasodilator response to bradykinin. The dog hindlimb preparation was used as a bioassay. Mongrel dogs were anesthetized and the femoral arteries were isolated and fitted with a noncannulating electromagnetic flow probe. An indwelling catheter was also placed for administration of saline, bradykinin, or the various analogs. The vasodilatory responses of the hindlimb circulation to bolus doses of bradykinin from 1 of 20 ng were tested during vehicle or analog administration at 1 and 10 μg/min. Bradykinin analogs which were characterized by amino acid replacement by β-(2-thienyl)-L-alanine (Thi) at positions 5 and 8, D-phenylalanine (D-Phe) at position 7, and an additional replacement of hydroxyproline at position 2 or 3 were effective antagonists of bradykinin. The decapeptide bradykinin analog (BKA06) D-Arg-(Hyp^3-Thi^5-D-Phe^7-Thi)-BK was the most potent analog tested, producing a full log dose shift in the dose–response curve to bradykinin at the 10 μg/min (4 nmole/min) infusion rate. None of the analogs we tested produced vasodilation or had any effect upon systemic blood pressure at the concentrations tested. Our results suggest that these structural analogs of bradykinin may be effective pharmacologic tools to study the role of endogenous kinins in the control of vascular resistance and circulatory homeostasis.

Recently, it has been reported (1) that a number of structural analogs of the bradykinin molecule may act as sequence-related competitive antagonists of bradykinin. These observations have been made in various *in vitro* bioassays, including rat uterus and guinea pig ileum. The key to successful antagonistic properties seems to involve the replacement of the proline residue at position 7 by D-phenylalanine (1) or by other small D-aromatic amino acid residues (2, 3). Recently, one such analog has been shown to cause a transient rise in blood pressure when given *in vivo* (4). The potential of these compounds as research tools in studying the physiological actions of endogenous kinins led us to test the efficacy of several analogs to inhibit the vasodilator response to bradykinin in the canine femoral artery. In this study we have compared the relative antagonistic properties of six different bradykinin analogs to the vasodilatory activity of exogenous bradykinin in the canine hindlimb *in vivo*.

Methods. After overnight fasting, six female mongrel dogs were anesthetized with pentobarbital (25 mg/kg) diluted 1:2 with normal saline and placed on their backs to expose the inner legs. They were ventillated mechanically with a Harvard respirator (Harvard Instruments, MA) based upon the nomogram of Kleinman and Radford (5). Body temperature was maintained with a Hamilton Aquamatic K heating pad (Hamilton).

A catheter (PE160) was placed in the left femoral artery to continuously monitor blood pressure using a Statham P23Db pressure transducer connected to a Brush 440 recorder. The left femoral vein was catheterized (PE160) for periodic supplementation of pentobarbital anesthesia and for a maintenance infusion of 0.9% saline at 1.23 ml/min. The right femoral artery was exposed and fitted proximally with a noncannulating electromagnetic flow probe (Carolina Electronics, Burlington, NC) attached to a flowmeter (Carolina) and the recorder. An infusion catheter (PE90) with a three-way valve was fitted into the artery via a small arterial branch, proximal to the flow probe. A continuous infusion of 200 μl/min of saline was administered through this line. The third (side arm) branch of the line was

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attached to a siliconized glass gas-tight 1-cc syringe, and when not in use this line was occluded with shielded hemostats. The side-arm line was used to deliver individual 0.5-ml bolus injections of bradykinin or its saline vehicle. A 1 mg/kg (body wt) bolus of captopril (6) was administered by means of the femoral catheter to decrease kininase II activity. The dog was then allowed to stabilize for 20–30 min.

A dose–response curve to bradykinin acetate (Bachem Inc., Torrance, CA) was carried out with sequential doses of bradykinin delivered in 0.5 ml saline, including vehicle injection and amounts of bradykinin ranging from 1 to 20 ng. After completion of the bradykinin dose–response cycle, a bolus of 10 ng acetylcholine (Sigma, St. Louis, MO) in 0.5 ml was administered as a nonkinin vasodilator control. Following 10 min of recovery, infusion of one of six different bradykinin analogs was started in the femoral artery at a rate of 1 μg/min. After 3 min, the dose–response cycle to bradykinin was repeated in the presence of the analog. The analog infusion was then terminated, and saline infusion was resumed. After 10–20 min, the dose–response cycle with bradykinin was repeated. Then, the protocol was repeated using the same analog at a rate of 10 μg/min. Upon discontinuation of the analog, and after 20 min of saline infusion, the protocol cycle was repeated with different analogs.

At the conclusion of each experiment, the femoral artery was occluded and cannulated with a PE240 line. Timed blood collections were carried out to calibrate the flow probe. All responses are presented in absolute milliliters per minute of blood flow.

All bradykinin analogs were synthesized in the biochemistry laboratories of the University of Colorado. Based upon previous *in vitro* experience (1) and preliminary screening in a rat model (unpublished), six different analogs of bradykinin were chosen and screened using three repetitions of the experimental protocol in different dogs. Each dog was used to test more than one analog. The sequences of analogs administered and the sequences of doses were randomized. Sufficient time for complete reversal of the effects of each analog (approximately 10–20 min) was allowed and confirmed with a control bolus of bradykinin.

The six analogs were derived from the native bradykinin molecule by progressively replacing strategic amino acids at positions 7, 5, 8, 3, and 2. The complete structures of the native bradykinin molecule and the analogs are detailed in Table I. For the sake of simplicity,these bradykinin analogs (BKA) have been assigned names as follows: BKA01 (mol wt 1400), BKA02 (mol wt 1860), BKA03 (mol wt 1530), BKA04 (mol wt 1645), BKA05 (mol wt 2170), and BKA06 (mol wt 2360).

The analog which had the greatest antagonism of the vasodilator response to bradykinin (BKA06) was further characterized by expanding the doses of bradykinin used (0.5 to 30 ng) and increasing the number of dogs studied to five.

The effectiveness of each analog was tested by regression analysis of the data obtained in each dog using a logarithmic transformation of the linear portion of each dose–response curve. Comparison of the means of the calculated slope and *y*-intercept derived from the regression analysis was used to establish (respectively) parallelism of the dose–response and whether each analog caused a significant shift (attenuation of the vasodilator response

TABLE I. STRUCTURE OF NATIVE BRADYKININ AND THE SIX ANALOGS

	1	2	3	4	5	6	7	8	9
Bradykinin	Arg	Pro	Pro	Gly	Phe	Ser	Pro	Phe	Arg
BKA01	Arg	Pro	Pro	Gly	Phe	*D-Phe*	CDF	Phe	Arg–HOAC
BKA02	Arg	Pro	Pro	Gly	Phe	Ser	*D-PAL*	Phe	Arg–TFA
BKA03	Arg	Pro	Pro	Gly	*Thi*	Ser	*D-Phe*	*Thi*	Arg–TFA
BKA04	Arg	Pro	*Hyp*	Gly	*Thi*	Ser	*D-Phe*	*Thi*	Arg–TFA
BKA05 *Lys-Lys*	Arg	*Hyp*	Pro	Gly	*Thi*	Ser	*D-Phe*	*Thi*	Arg–TFA
BKA06 *D-Arg*	Arg	Pro	*Hyp*	Gly	*Thi*	Ser	*D-Phe*	*Thi*	Arg–TFA

Note. HOAC, acetic acid salt; TFA, trifluoroacetic acid salt; CDF, *p*-chloro-D-phenylalanine, Thi, β-(2-thienyl)-L-alanine, Hyp, L-4-hydroxyproline, Pal, β-(2-pyridyl)-L-alanine.

to bradykinin) of the dose–response curve. These analyses were run by *t* tests comparing the vehicle control of each dose–response to the response with either the 1- or 10-μg infusion rate of each analog. A *P* value (using an adjusted α-level to reflect multiple comparisons with control) of less than 0.025 was considered to be statistically significant. The extent of antagonism of each of the six analogs was standardized and evaluated by comparing the dose of bradykinin required to produce a mean 100 ml/min increase in femoral blood flow. A shift in the sensitivity of flow to bradykinin was expressed as a percentage of the paired response in the absence of the analog, where 1000% represents a shift in sensitivity to bradykinin of one complete log dose. The differences between analogs were evaluated by analysis of variance and a Scheffe test.

Results. The mean body weight of the six dogs studied was 21.2 ± 1.8 kg, the mean arterial pressure was 121 ± 6 mm Hg, and the absolute femoral blood flow equaled 87 ± 17 ml/min. Captopril had a mild and transient hypotensive effect.

The relative potencies of the six analogs are presented in Table II. None of the six analogs had any effect on systemic arterial pressure or upon the basal femoral vascular resistance. Neither BKA01 nor BKA02 (Fig. 1) demonstrated any effect upon the responsiveness to bradykinin at either 1 or 10 μg/min. In fact, BKA01 had a (nonsignificant) tendency to potentiate the vasodilator response to bradykinin.

TABLE II. THE CHANGE IN SENSITIVITY (% SHIFT) OF FEMORAL BLOOD FLOW TO EXOGENOUS BRADYKININ

Analog	Percentage shift (1 μg/min)	Percentage shift (10 μg/min)
BKA01	91 ± 12	69 ± 1
BKA02	100 ± 4	136 ± 6
BKA03	133 ± 4	260 ± 18
BKA04	159 ± 4*	371 ± 16*
BKA05	172 ± 8*	427 ± 12*
BKA06	265 ± 10*	1028 ± 77*

Note. Shift (as %) in the dose of bradykinin required to induce a 100 ml/min increase in femoral blood flow during administration of bradykinin analogs. Each of six analogs was administered at 1 and 10 μg/min. Values are expressed as a percentage of control, where 100% means no shift in the dose response and 1000% equals a full log dose shift in the dose–response curve. Asterisks denote a significant change ($P < 0.01$) in the dose–response compared with that of the control.

The analogs BKA03, BKA04, BKA05, and BKA06 (Figs. 1 and 2) demonstrated inhibition of bradykinin. Although the changes produced by analog BKA03 did not achieve statistical significance, the three analogs BKA04, BKA05, and BKA06 all produced significant and parallel shifts in the femoral vasodilator response to bradykinin (Figs. 1 and 2) as summarized in Table II, representing a significant antagonism of bradykinin. Analogs BKA04 and 05 produced statistically similar responses. At the higher 10 μg/min infusion rate, BKA04 caused approximately one-third of a log dose shift and BKA05 caused almost one-half of a log dose shift. Analog BKA06 (Fig. 2) exhibited significantly greater antagonism than all other analogs tested. The 1 μg/min dose caused over one-quarter of a log dose shift in the vasodilator response to bradykinin, and 10 μg/min shifted the femoral vasodilator response to bradykinin over a full log dose (Table II). This analog was more than twice as effective as any of the others tested. Since this analog also has a greater molecular weight, on a molar basis it is even more effective than the other analogs.

The vasodilatory response to acetylcholine was unaffected by 10 μg/min of any analog. Also, the reversibility of all analogs was tested by repeated challenges with 2.5 ng bradykinin over 20 min of recovery after conclusion of the analog infusion. We found complete restoration of the vasodilatory response to bradykinin within 10–12 min after cessation of each analog.

Discussion. The possibility that the vasoactive kinins participate in the intrinsic regulation of peripheral circulation and cardiovascular homeostasis has been hypothesized (7). However, development of a definitive role of kinins has been hindered by the lack of experimental pharmacologic probes by which to study the mechanisms of the kinins' action. Recently (1), a series of structural analogs of bradykinin which possess competitive antagonistic properties to exogenous bradykinin when used with *in vitro* bioassays has been reported. Recently, Benetos *et al.* (4) found that a 1-mg bolus injection (*in vivo*) of the analog BKA04 caused a transient rise in systemic blood pressure in the rat, but that smaller doses

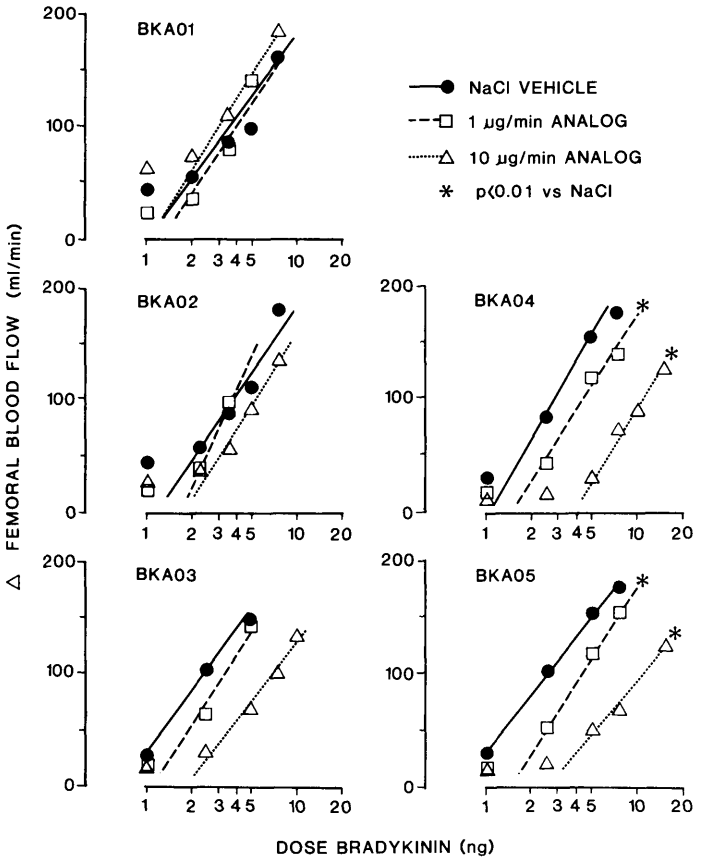

FIG. 1. Dose–response curves to graded doses of bradykinin between 1 and 15 ng during saline infusion or administration of 1 or 10 μg/min of one of five different bradykinin analogs. The linear portions of all dilator–response curves were parallel. Significant shifts in the dose–response curves ($P < 0.01$) compared with that of control are indicated by asterisks at the zeniths of the curves.

had no effect. In another study (8), this analog was found to partially inhibit the vasodepressor effect of exogenous bradykinin and to partially reverse the antihypertensive effect of converting enzyme inhibition. While these reports are suggestive of a role of bradykinin in the control of blood pressure, the studies did not systematically test the efficacy of the analog BKA04, nor did they test the potency of other similar analogs in blocking the vascular effects of bradykinin.

Our studies are the first to report the relative efficacy of these compounds upon the vascular resistance responses to graded doses of bradykinin. Our results suggest that key replacement with the amino acids Hyp^3, Thi^5, D-Phe^7, and Thi^8 results in significant antagonism of the vasodilatory action of kinins. Further, none of the kinin analogs tested induced vasodilation or altered blood pressure under these experimental conditions. It has been previously reported that single substitution in position 7 produces analogs with little agonistic activity in the uterus assay or upon rat blood pressure (1). Only analogs with a D-Phe^7 substitution were thought to be antagonistic in the ileum assay (1), but additional small D-aromatic amino acid residues at position 7 also confer antagonistic activity (2, 3). All of the analogs we have tested which have exhibited antagonistic properties in the canine hindlimb have the proline of position 7 replaced by another amino acid. However, this substitution was not sufficient alone to produce good antagonistic properties in the hindlimb preparation. BKA01, BKA02, and BKA03 all had

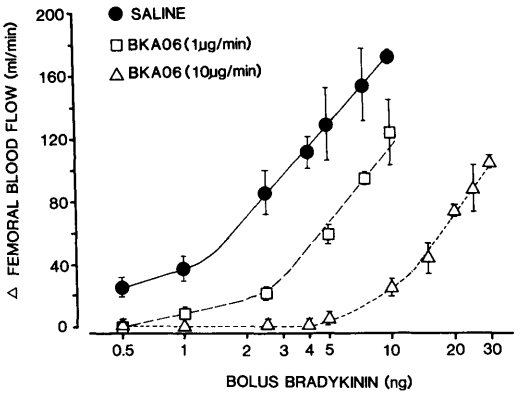

FIG. 2. Dose-response curves to graded doses of bradykinin between 0.5 and 30 ng during saline infusion or administration of 1 or 10 μg/min of the bradykinin analog BKA06. The linear portions of all dilator-response curves are parallel, and each concentration of analog produced a significant shift of the linear portion of the curves compared to that of the control ($P < 0.01$).

substitutions in position 7, and yet did not achieve significant antagonism of the response to exogenous bradykinin.

Previously, the most potent analog agonist to bradykinin in smooth muscle preparations had replacements at positions 5 and 8 with the isoteric thienylalanine (9) which led to increased receptor affinity in the uterus assay (10). Combination of the replacements at positions 5, 7, and 8 led to potent antagonistic properties in both uterus and ileum with no agonistic activity in the rat blood pressure assay (1). This stuctural combination also provides the basis for effective antagonism of bradykinin-induced vasodilation in the hindlimb. However, the addition of L-4-hydroxyproline at position 2 (BKA05) or 3 (BKA04 or BKA06) has amplified the antagonistic properties of these analogs in our preparation. Addition of amino acids at the amino terminal (BKA05 and BKA06) also increased the antagonism of bradykinin-induced vasodilation in the hindlimb. However, at concentrations which significantly altered the response to large doses of bradykinin in the hindlimb, we observed no effects upon systemic blood pressure or even the basal vascular resistance within the vasculature of the hindlimb. It is of considerable interest that we found BKA06 to be approximately three-fold more potent an antagonist than BKA04, which was the compound used by Benetos *et al.* (4, 8) to suggest a role for bradykinin in blood pressure homeostasis.

This characterization of significant antagonistic properties of a family of analogs of bradykinin *in vivo* suggests that these compounds will be useful tools for further investigation of the biological role of the kallikrein-kinin system. The analog BKA06 seems particularly good as an antagonist of the effects of kinin upon the peripheral vasculature.

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