Biological Activities of Photoaffinity Labeling Analogues of Kinins and Their Irreversible Effects on Kinin Receptors

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Several analogues of bradykinin and [des-Arg^9]bradykinin with potentially reactive groups have been tested for their biological activities. In these analogues, phenylalanine was replaced by the aromatic amino acid (4′-nitro)Phe, (4′-amino)Phe, (4′-azido)Phe, and (4′-diazonium)Phe, as well as with other residues. [Des-Arg^9]bradykinin and the octapeptide analogues were tested on rabbit aorta strips, an assay organ containing the B_1 receptor which is activated by the octapeptide [des-Arg^9]bradykinin. Strips of rabbit jugular vein served as bioassays for bradykinin and the nonapeptide analogues, because the rabbit jugular vein bears the receptor B_2 which is sensitive to bradykinin nonapeptides. The biological findings support the interpretation that kinins act on two different receptor types, since the potency orders of the analogues in the two bioassays are different. All potential photolabels retained reasonable affinities in the dark, except [(4′-azido)Phe^8,des-Arg^9]bradykinin, which, however, proved to be a weak and competitive antagonist of [des-Arg^9]bradykinin on the rabbit aorta. Inactivation experiments with the unstable (4′-diazonium)Phe-containing peptides did not show any irreversible effect in the two bioassays. Photoaffinity labeling experiments with the azido and the nitro peptides gave irreversible and specific effects on both the rabbit aorta and the jugular vein. [(4′-Azido)Phe^8,des-Arg^9]bradykinin photolyzed at 365 nm on the rabbit aorta reduced the sensitivity of this tissue against [des-Arg^9]bradykinin specifically to about one-third of its initial value. [(4′-Azido)Phe^5]bradykinin reduced the sensitivity of the rabbit jugular vein to bradykinin by more than 50%. The observed irreversible effects were always loss of myotropic activity and never permanent contraction.

The peptides of the kinin family, such as kallidin (KD) and bradykinin (BK), have received much attention in recent years because of their implication in pathophysiological conditions, like inflammation, hypertension, and pain.[1] Many attempts have been made to find specific competitive antagonists for the kinins in order to characterize the receptors and, in a second step, for medical applications. Recent advances have shown that BK (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) and KD (Lys^0-BK) have fundamentally different actions as their [des-Arg^9]BK and [des-Arg^{10}]KD fragments.[2] Specific and competitive antagonists are known for B_1 receptors, while such compounds are not available for B_2 receptors. The different actions and pharmacological properties of BK and its analogues and the analysis of the different bioassays have been extensively reviewed recently.[1] The receptors for kinins, namely the B_1 receptor of the rabbit aorta and the B_2 receptor of other tissues, specifically the rabbit jugular vein, have not been purified from their target tissues. This might well be only possible with covalent labeling techniques, because receptors for kinins may not retain binding capacity if the embedding cell membrane is destroyed, i.e., by solubilization with a detergent. If this kind of receptor can be labeled specifically and covalently in its original environment, then the cell membrane can be solubilized followed by biochemical isolation of the labeled protein.

Potential irreversibly acting analogues of bradykinin have been prepared by several research groups,[3,4] and irreversible effects have, indeed, been observed.[5] However, the chemical instability to water and other nonspecific nucleophiles requires high concentrations of label in solution and produces solubility problems. The labels also inactivate themselves by intra- and intermolecular nucleophilic attack, e.g., through arginine and serine side chains and through water of hydration. This makes it necessary to perform synthesis immediately before use. For the same reason, it is almost impossible to produce and apply radioactively marked labels with sufficiently high specific activity for the detection of a covalently labeled receptor. The needed minimal specific activity for detection of the label is several curies per mmole[6] which gives, for the high concentrations needed, inconveniently high levels of radioactivity (e.g., M_r 1000; 30 Ci/mmol, 1 mg contains already 30 mCi!). Nevertheless, these affinity labels (BK analogues substituted with chloromethyl ketones, active esters, maleimides, and mustards) are very useful pharmacological tools for studies in vitro and in vivo.

We therefore decided, encouraged by the earlier success with angiotensin II,[7] to apply the photoaffinity labeling technique on the receptors of the kinins. With this technique, highly active and chemically stable peptides containing sufficient radioactivity are feasible.[6] These labels can bind competitively to their receptors in the dark, and upon activation by long-wavelength ultraviolet light, this binding becomes irreversible (covalent). Recently, we have published[8,9] the synthesis of such potential affinity and photoaffinity labeling analogues of BK and [des-Arg^9]BK (see Table I). The 4′-nitrophenylalanyl peptides and the 4′-amino-3′,5′-diiodophenylalanyl analogues have been obtained by classical solid-phase synthesis. All other peptides were produced by modification of these precursors, and their structures are presented in Chart I. The only exception was the 3′-[(4″-azidophenyl)azo]tyrosine-containing analogue, which was obtained by coupling [Tyr^5,des-Arg^9]BK with diazotized *p*-azidoaniline (Chart I).[9] Such an array of analogues is perfectly suited for structure–activity studies of an aromatic position in a peptide hormone.[10,11]

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Table I. Biological Activities of Bradykinin Octa- and Nonapeptides

no.	peptide[a]	ED_{50}[b]	pD_2[c]	RA[d]	α^E[e]
1	OBK (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe)	67.98	7.16	100	1
2	[(4′-NO_2)Phe^5]OBK	437 ± 168	6.36	16	1
3	[(4′-NH_2)Phe^5]OBK	5464 ± 1600	5.26	1.2	1
4	[(4′-N_2^+)Phe^5]OBK	3753 ± 930	5.43	1.8	1
5	[(4′-N_3)Phe^5]OBK	461 ± 60	6.34	15	1
6	[(4′-OH)Phe^5]OBK = [Tyr^5]OBK	869 ± 150	6.06	8.0	1
7	[(4′-OH-3′-(4″-N_3-Ph-N_2))Phe^{5}¼OBK	1280 ± 450	5.89	5.4	1
	[Car^5]OBK		6.32[f]	14	0.76
	[(4′-OCH_3)Phe^5]OBK = [Tyr-OMe^5]OBK		5.27[f]	1.3	1
	[Cha^5]OBK		7.21[f]	110	1
	[Leu^5]OBK		5.81[f]	4.5	1
	[Ala^5]OBK		6.03[f]	7.0	1
8	[(4′-NO_2)Phe^8]OBK	$>10^5$	<4		
9	[(4′-NH_2)Phe^8]OBK	4647 ± 1070	5.33	1.5	1
10	[(4′-N_2^+)Phe^8]OBK	824 ± 290	6.08	8.3	1
11	[(4′-N_3)Phe^8]OBK	8556 ± 2300[g]	5.07[g]	0.8	0
	[(4′-OH)Phe^8]OBK = [Tyr^8]OBK		4.39[f]	0.2	1
	[(4′-OCH_3)Phe^8]OBK = [Tyr-OMe^8]OBK		4.28[f]	0.1	1
	[Car^8]OBK		<4[f]		
	[Cha^8]OBK		6.42[f,g]	18	0
	[Leu^8]OBK		7.27[f,g]	108	0
	[Ala^8]OBK		4.78[f,g]	0.40	0
12	BK (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg)	1.65	8.78	100	1
13	[(4′-NO_2)Phe^5]BK	24.0 ± 11	7.62	7	1
14	[(4′-NH_2)Phe^5]BK	57 ± 16	7.24	3	1
15	[(4′-N_2^+)Phe^5]BK	25 ± 5	7.73	9	1
16	[(4′-N_3)Phe^5]BK	10 ± 5	8.0	17	1
17	[(4′-NH_2-3′,5′-I_2)Phe^5]BK	$>10^5$	<4	<0.01	
	[Car^5]BK		5.68[f]	0.08	
	[(4′-OH)Phe^5]BK = [Tyr^5]BK		6.21[f]	0.3	1
	[(4′OCH_3)Phe^5]BK = [Tyr-OMe^5]BK		7.93[f]	14	1
	[Cha^5]BK		8.15[f]	23	1
	[Leu^5]BK		7.70[f]	8	1
	[Ala^5]BK		5.41[f]	0.1	
18	[(4′-NO_2)Phe^8]BK	6.6 ± 2.4	8.18	25	1
19	[(4′-NH_2)Phe^8]BK	19.5 ± 2.5	7.71	9	1
20	[(4′-N_2^+)Phe^8]BK	25 ± 5	7.73	9	1
21	[(4′-N_3)Phe^8]BK	7.0 ± 2	8.15	23	1
22	[(4′-NH_2-3′,5′-I_2	$>10^5$	<4	<0.01	
	[Car^8]BK		5.42[f]	0.04	
	[(4′-OH)Phe^8]BK = [Tyr^8]BK		7.34[f]	3.5	1
	[(4′-OCH_3)Phe^8]BK = [Tyr-OMe^8]BK		8.59[f]	65	1
	[Cha^8]BK		8.15[f]	23	1
	[Leu^8]BK		5.77[f]	0.1	1
	[Ala^8]BK		4.37[f]	0.01	1

[a] OBK = [des-Arg^9]bradykinin; Car = carboranylalanine; Cha = cyclohexylalanine. [b] ED_{50} is the nanomolar concentration of peptide producing 50% of the maximal response. [c] pD_2 is the negative logarithm of the molar concentration of peptide producing 50% of the maximal response. [d] RA is the relative affinity in percent of that of the parent agonist (BK or OBK). [e] α^E is the intrinsic activity. [f] Values are from the literature. [g] Indicates that the compound is an antagonist; their ID_{50} is the nanomolar concentration of antagonist which reduces the response of a double dose of agonist to that of a single dose. The value listed in the pD_2 column is the pA_2 value and, for the sake of simplicity, compared to the pD_2 values. pA_2 = −log of ID_{50} expressed as molar concentration. OBK and analogues are measured on rabbit aorta strips; BK and nonapeptide analogues are determined on rabbit jugular vein.

Important differences in the structure–activity relationships on Phe in position 8 of BK and [des-Arg^9]BK has principally helped to distinguish between B_1 and B_2 receptors. However, the direct influence of the aromatic side chains on the hormone–receptor interaction is still poorly known and needs further investigation. The aim of this study is, therefore, twofold: (a) to analyze the biological activities of the newly prepared kinin analogues in the dark in view of determining the function of the aromatic residues in the hormone–receptor interaction. This might help to determine the physicochemical property of an aromatic side chain which contributes to the binding process or even to receptor stimulation. (b) To find agents which are capable of blocking receptors B_1 and B_2 for kinins permanently and specifically. Such a compound could be used as a specific blocker for pharmacological characterization of receptors and in a parallel study with the radioactive label for the biochemical isolation of such receptors.

Structure–Activity Studies. BK and BK–nonapeptide analogues were tested in vitro on strips of rabbit jugular vein; [des-Arg^9]BK and the corresponding octapeptide analogues were tested on rabbit aorta strips. In these first experiments, all compounds were merely tested for their agonistic properties without looking for potential irreversible effects. All compounds except 7 were tested in the absence of ultraviolet radiation (incandescent illumination only), and compound 7, which absorbs visible light, was tested under darkroom conditions. The biological activities are expressed by the concentration of each peptide required to produce a half maximal effect (pD_2 value) and the derived relative affinity in percent of that of BK and [des-Arg^9]BK; the maximum stimulation capacity is expressed by the intrinsic activity α^E. The results are presented in Table I and compared to those obtained with other, already described analogues.[1] Since the main purpose of this study was not to prove the difference of

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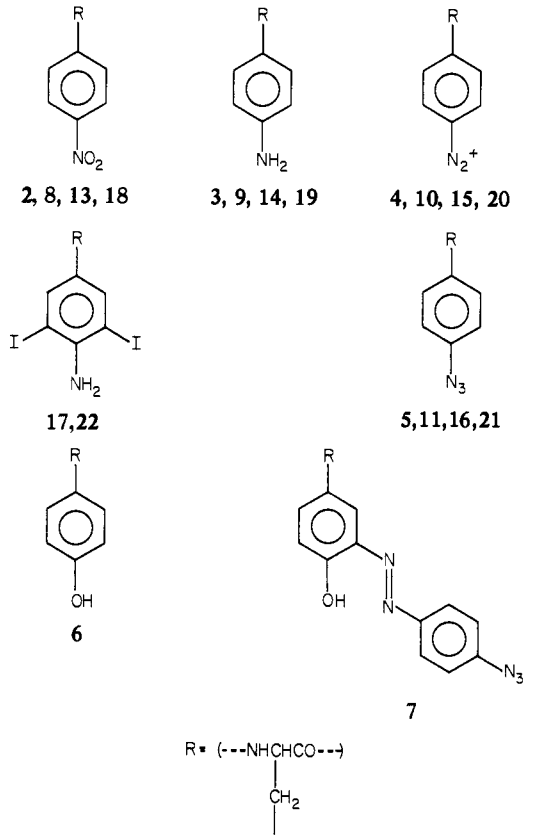
Chart I. Chemical Structures of Phenylalanines



the postulated B_1 and B_2 receptors for kinins but rather to find or identify potential irreversible analogues of these peptides, no crossover study was done with B_1 agonist on tissues containing receptor B_2 and vice versa (in other words, BK nonapeptides were not tested on the rabbit aorta and the octapeptides were not on the jugular vein).

From the results presented in Table I, the following considerations can be made regarding the relative functional roles of the two aromatic residues Phe[5] and Phe[8] in both the nonapeptide and octapeptide structures.

Position 5. The octapeptides with modification in position 5 display on the B_1 receptor of rabbit aorta a potency order which is different from the potency order of nonapeptides on the B_2 receptor of the rabbit jugular vein: large side chains on the octapeptides do not interfere seriously with the relative affinity, even very large chains such as in analogues **7** or [Car[5],des-Arg[9]]BK. On the other hand, small and polar modifications on the side chain, such as in **3** and [Tyr[5],des-Arg[9]]BK, induce a similar loss of affinity. The difficulty in comparing the data of the octapeptides with those of the nonapeptides derives from the sensitivity differences in the two assay systems: the rabbit jugular vein is at least one order of magnitude more sensitive than the rabbit aorta. The jugular vein receptor B_2 appears to be less tolerant to change in the size of the side chain in position 5: both a reduction of the size (e.g., [Ala[5]]BK) and an increase of the size (e.g., [Car[5]]BK and **17**) significantly reduce the biological activity. The potential label **16** retains some reasonable affinity which is advantageous for the studies that follow. It seems that steric parameters are the most important for this position in the nonapeptide structure because side chains which are too small or too large gradually loose affinity: [Ala[5]]BK < [Leu[5]]BK < **12** > **13** > [Car[5]]BK > **17**. Aromaticity is probably not essential; the cyclohexylalanine analogue retains more than 20% of the affinity of the parent peptide.

Position 8. The only important new information concerning this position comes from the octapeptide–B_1 system. Until now it was believed that only aliphatic side chains in position 8 can produce antagonists and that the chemical group mediating the receptor activation is the aromatic side chain of Phe 8. However, **11** [(4′-azido)Phe[8] octapeptide] is a weak but pure antagonist up to concentrations of 100 μM. This means that reasons other than the lack of aromaticity have to be considered in order to account for antagonism, a situation which is very similar to what has been observed with angiotensin II.[10,11]

On the nonapeptide–B_2 system, no antagonists are found, but again the size of the side chain seems to influence the affinity: [Ala[8]]BK and **22** are practically inactive. Fortunately, the azide compound **21** retains good affinity for potential labeling purposes.

A more detailed study of these side-chain parameters could perhaps shed more light on the conditions for optimal receptor binding, stimulation, or inhibition.

Inactivation Experiments. In the foregoing chapter the analogues described in Table I were merely tested for their myotropic actions on smooth-muscle preparations; no mention was made about the duration and reversibility of their actions. Two families of kinin analogues with potentially irreversible character had to be tested for prolonged action: photosensitive analogues and diazonium peptides. The photosensitive nitro and azido peptides are chemically stable compounds, but under ultraviolet irradiation they form highly reactive intermediates; the diazonium peptides are stable for a short time at pH ~2 but highly reactive at physiological pH. They all could theoretically react with various protein side chains and form chemically stable links. If the stable nitro and azido compounds were tested in the absence of ultraviolet light, no long-lasting effects were observed, as expected. The tissues recovered full sensitivity against the parent hormones (BK and [des-Arg[9]]BK, respectively) after removal of each analogue.

The diazonium compounds, however, are potential affinity labels, capable of binding covalently to several natural amino acid side chains found in proteins (for instance, the side chains of tyrosine or of histidine). The diazonium peptides had to be prepared immediately before use from the (4′-amino)Phe analogues under very acid conditions (pH ~1) because diazonium salts can react intra- and intermolecularily. As was observed with angiotensin II,[11] no long-lasting or irreversible effects were visible upon addition of these diazonium peptides of the kinin series to the tissues. All treated tissues retained full sensitivity against BK or [des-Arg[9]]BK, respectively, even after repeated incubations with the diazonium peptides.

The irradiation of tissues in the presence of azido and nitro peptides produced in some cases specific and irreversible partial blocks of the receptor targets (see Table II), similar to the results obtained with angiotensin II.[7] The specificity of this inactivation has been assessed by the following controls: (1) in parallel experiments without ultraviolet irradiation, the labeling peptides did not display any long or irreversible action. (2) The tissue response to other myotropic agents did not change during the experiment; the sensitivity against nonkinin agents was the same after the photolabeling treatment as it was before. Angiotensin II and noradrenaline were used on the rabbit aorta and histamine and substance P on the rabbit jugular vein for this purpose. (3) The presence of (4′-amino)-phenylalanine in the photolabeling experiment did not prevent the observed inactivation. This compound was added as a scavenger in order to prevent nonbound and

Table II. Inactivation Experiments [a]

bioassay	treatment	dose, μM	no. of exposures	residual response, [b] %	competitive protection
rabbit aorta with octapeptide analogues	light only		2	NE; 95 ± 5	
	light + 1	10	2	NE; 85 ± 7	
	light + 2	10	2	NE; 92 ± 7	
	light + 8	10	2	76 ± 7	75 ± 7
	light + 5	10	2	NE; 90 ± 6	
	light + 11	10	2	37 ± 3	95 ± 8
	light + 7	20	1	69 ± 10 [c]	not tested [c]
	4 only	40	2	NE; 98 ± 3	
	10 only	70	2	NE; 95 ± 4	
rabbit jugular vein with nonapeptide analogues	light only		3	NE; 93 ± 7	
	light + 12	0.1	3	NE; 92 ± 6	
	light + 13	0.5	3	NE; 88 ± 8	
	light + 18	0.1	3	NE; 91 ± 5	
	light + 16	1.0	3	44 ± 13	91 ± 5
	light + 21	0.2	3	NE; 85 ± 7	
	15 only	3.0	2	NE; 92 ± 6	
	20 only	2.0	2	NE; 94 ± 5	

[a] All experiments are at least triplicate plus or minus SE. If less than 25% inactivation was observed, the compound was considered ineffective. [b] NE = no effect. [c] This compound was tested in triplicate under darkroom conditions. Due to th the high concentrations needed and the small quantities of 7 available, no competitive protection could be tested and only one treatment was carried out.

light-activated photolabel to diffuse to the tissue and damage it by nonspecific attachment; any free and reactive substance would be intercepted by this scavenger. Therefore, we have good reasons to assume that in a first time the photolabel was first reversibly bound to the receptor and then it was activated by light in situ and reacted with the receptor. (4) If a considerable inactivation of the [des-Arg^9]BK or the BK response was observed (see Table II), the experiments were repeated, but this time the tissues were treated prior to labeling with large doses of a specific and nonphotolyzable analogue. This competitive protection prevented any inactivation of the receptors, and tissue sensitivity against [des-Arg^9]BK and BK, respectively, was fully recovered. (5) If prephotolyzed label was used in an experiment instead of the label, again no inactivation was observed. This indicates further that the initial photoproduct of the label carries out the inactivation. In the case of the azido compounds, this probably means that the nitrene or a reactive and short-lived intermediate or rearrangement product forms a covalent link with the receptor. As stated earlier, the situation for the nitro compounds is not clear, and the still hypothetical but effective mechanism[7] of the nitrophenylalanine photolysis awaits further clarification. However, no nitro compounds gave any reasonable inactivation of the kinin receptors, and the effect of 8 is weak and nonspecific because it is not prevented by competitive protection. (6) The inactivated tissues did not recover their BK or [des-Arg^9]BK sensitivity during a 12-h period. We did not expect 100% inactivation of the receptors of BK and [des-Arg^9]BK with our treatments. Due to the chemical nature of the labeling moiety it is not possible to obtain 100% insertion even with repeated treatments, and no such yield is reported in the literature. However, labeling only a few percent is sufficient for a successful isolation of receptor proteins, provided a label with sufficiently high specific radioactivity is utilized.

From the results presented in Tables I and II it can be seen that the [des-Arg^9]BK receptor B_1 is differently organized from the BK receptor B_2: (A) The potency order of the described octapeptide analogues is very different from the potency order of the nonapeptide analogues. (B) The receptor B_2 inactivation is achieved with the BK analogue containing (4-azido)Phe in position 5, while the best result with the octapeptides is obtained with the analogues modified in position 8.

It is important to note that the biological response to a permanently occupied receptor is not, as expected, permanent stimulation but rather permanent loss of sensitivity, as if a permanent tachyphylaxis had developed. To date, all studies made on smooth-muscle systems with irreversibly acting compounds show the same blocking effect.[5,7,12] On the other hand, similar studies on secretory systems always produce permanent stimulation.[13-15] The following questions arise from these results: (a) Are secretory receptors different from myotropic receptors? A study with irreversible analogues of a hormone with multiple functions is needed, provided there is enough evidence to assume that the receptors are identical. Angiotensin II is becoming such a case where evidence is accumulating that smooth-muscle receptors are at least very similar, if not identical with adrenal cortical receptors.[16-18] However, for the kinins no true secretory bioassay is known and, therefore, the answer to those questions will probably not be found with photolabile kinin analogues.

The synthesis of radioactive photolabile kinin analogues starting with the peptide 17 using already described procedures[6] and the labeling of kinin receptors are currently under investigation. Such a study will probably yield additional evidence for the differentiation of B_1 and B_2 receptors for kinins.

Experimental Section

Bioassays. Biological activities of the tissues in response to BK, its analogues, and other agonists were recorded with force-displacement transducers (Grass FT 03) on a Grass polygraph

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Model 7 (Grass Co., Quincy, MA). All experiments were performed in the absence of ultraviolet radiation,[7] except when indicated. For the tissue preparation, albino rabbits (New Zealand) of either sex, weighing 1.5–2.0 kg, were killed by stunning and exsanguination. The thoracic aorta and the jugular vein were dissected away, freed from connective tissue and fat, cut into helical strips 2- to 2.5-cm long, and then suspended in 5-mL organ baths containing oxygenated (95% O_2 and 5% CO_2) Krebs' solution at 37 °C. The peptides used were BK, [des-Arg^9]BK, and their respective analogues, [Sar^1]angiotensin II, [Nle^{11}]substance P, [Sar^1,Leu^8]angiotensin II, [Leu^8,des-Arg^9]BK (all from our laboratory), and the protease inhibitor SQ 14225 (Squibb & Sons Inc.). The other drugs used were histamine, noradrenaline, cycloheximide (all from Sigma), and the scavenger 4′-aminophenylalanine (our laboratory). Concentrated solutions (1 or 5 mg/mL) of the drugs were prepared in distilled water and diluted with 0.9% saline to the desired concentrations. Solutions of noradrenaline were acidified with 0.01 N HCl. Rabbit aorta strips were equilibrated during 3 h until a good sensitivity to [des-Arg^9]BK developed; from this point on, the tissues were permanently treated with cycloheximide (10 μg/mL) in order to prevent the formation of additional B_1 receptors.[2] Strips of jugular veins were equilibrated during approximately 2 h and kept in contact with the protease inhibitor SQ 14225 (100 ng/mL) in order to enhance and stabilize the responses of BK and its analogues.[1] The biological activities of the described analogues of [des-Arg^9]BK on the rabbit aorta were determined with cumulative concentration–response curves, and their specificity was demonstrated by the block with a specific inhibitor ([Leu^8,des-Arg^9]BK) of B_1 receptors. Since inhibitors are not available for B_2 receptors, only desensitization experiments were performed with BK analogues in jugular veins desensitized with BK or with one of the analogues.

Inactivation Experiments. The irradiation equipment with mercury lamps has already been described,[7] and produced, around λ 365 nm, a mean radiation density of 18 mW/cm^2 on the tissue, measured with a Spectroline radiometer DM 365 N (spectronics Corp., Westbury, NY). In a typical experiment, the tissues were washed every 15–20 min with fresh Krebs' solution. After the incubation period, the tissues were challenged twice with BK (jugular vein) or [des-Arg^9]BK (aorta) at 60-min intervals and washed after the maximal response was obtained. After relaxation to the base line, the sensitivity of the tissues to other agents was tested: that of the jugular vein with 1 μM [Nle^{11}]substance P and 7 μM histamine; that of the aorta with 3 μM noradrenaline and 0.2 μM [Sar^1]angiotensin II. After these activity tests, the tissues were incubated with the scavenger (4′-amino)Phe (100 μM) and the kinin analogue under assay. At maximal contraction or 5 min after addition of the analogue, in the cases where no contraction developed the tissues were exposed to the light source for 10 min and washed thoroughly afterwards.

The diazonium peptides were prepared immediately before use in the following manner: 100 μg of the (4′-amino)Phe-containing peptide (**3**, **9**, **14**, and **19**) was diazotized in 470 μL of 0.1 N HCl at 0 °C with 10 μL of a 1 N $NaNO_2$ solution. After 5 min and a positive iodine–starch test, 20 μL of a 1 N solution of sulfamic acid was added, in order to destroy excessive nitrite. If a second iodine–starch test was negative, the solution was used directly for the pharmacological tests. The buffer capacity of the tissue baths solution was strong enough to absorb the added acid. The schedule of the inactivation experiments with the diazonium peptides was the same as shown for the photosensitive peptides but without irradiation and scavenger. For every experiment, one control tissue was exposed only to BK or [des-Arg^9]BK, and one tissue was exactly treated as above, except for light, and served as standard. The treatment was repeated once or twice as indicated in Table II on the same tissue. Sixty minutes after the last treatment, the tissues were tested for their residual activity against the standard agonists (BK or [des-Arg^9]BK) and the control agents (histamine and [Nle^{11}]substance P or noradrenaline and [Sar^1]angiotensin II). The photoinactivations given in Table II are the residual responses to BK or [des-Arg^9]BK compared to the simultaneous responses on reference tissues. If the reference tissues lost more than 25% of the initial sensitivity, the results were discarded. The residual activity of the treated tissues was redetermined every 45 min in order to detect slow recovery; up to 12 h after the beginning of an experiment, no such recovery was observed. No reduction of the response against the control agonists resulted from the treatment, indicating a specific action on kinin receptors only.

The successful inactivation experiments were repeated in the presence of 5 μM BK or 50 μM [Leu^8,des-Arg^9]BK, added prior to the photosensitive analogue and irradiation. This treatment successfully prevented inactivation and showed again the specificity of this inactivation. Prephotolyzed **8**, **11**, and **16** were used in preliminary experiments instead of the photolabels; again, no inactivation was observed when these compounds (prephotolyzed for 30 min in Krebs' solution containing the scavenger) were irradiated on the tissues.

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