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HYPOTENSIVE EFFECT OF LYS^9-BRADYKININ IN THE GUINEA PIG*

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Abstract—Lys^9-bradykinin is more potent than bradykinin in lowering the mean systemic arterial blood pressure of the guinea pig. Lys^{10}-kallidin is about as active as kallidin in this regard. The Lys^9 derivative is less potent than bradykinin in other tests or in other laboratory animals. The activity of the peptide increased when the guinea pigs were pretreated with mercaptoethanol. Intravenous injection of carboxypeptidase B blocked the effect of Lys^9-bradykinin in the guinea pig. The injected enzyme was removed by the kidney from the circulation and was excreted in the urine. Diluted guinea pig serum inactivated bradykinin and Lys^9-bradykinin approximately at the same rate.

SINCE 1960 more than one hundred analogues of bradykinin have been synthesized, but only a few of them are more active than bradykinin.[1, 2] Most of these peptide derivatives are less potent by orders of magnitude. Recently some synthetic analogues of bradykinin and kallidin were prepared,[1, 3–8] in which a terminal arginine of the peptides was replaced by another basic amino acid. Of these, Lys^9-bradykinin (lysine-^{9} bradykinin) and Lys^{10}-kallidin (Fig. 1) had but a fraction of the activity of bradykinin

H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Lys-OH
1 2 3 4 5 6 7 8 9

H-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Lys-OH
1 2 3 4 5 6 7 8 9 10

FIG. 1. Structure of Lys^9-bradykinin and Lys^{10}-kallidin.

in numerous tests. For example, Lys^9-bradykinin is 500 times less active than bradykinin on the isolated rat uterus, 5 times less on the guinea pig ileum, and 10–30 times less potent on the rabbit blood pressure.[1] Our studies with this peptide were prompted by an observation that, in contrast to the findings made in other animals or in other tests, Lys^9-bradykinin is significantly more hypotensive than bradykinin in the guinea pig.[2, 9]

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MATERIALS AND METHODS

In all, forty guinea pigs in sodium pentobarbital anesthesia (30–40 mg/kg) were used. Some of the animals were also pretreated with 10 mg atropine/kg i.p. The systemic blood pressure of the animals was recorded by means of a Statham pressure transducer connected to a Grass polygraph. The peptides were injected into a jugular vein usually at 15-min intervals. In five experimental animals the vagus nerves were cut.

The contractions of the isolated rat uterus *in vitro* were measured with a special device.[10]

Swine pancreatic carboxypeptidase B was prepared in this laboratory from acetone-dried swine pancreatic powder.[11] The activity of the enzyme was determined with hippuryl-L-arginine substrate either in an ultraviolet spectrophotometer[11, 12] or by means of a photometric ninhydrin assay method. The effect of injected carboxypeptidase B on kinins was established by comparing the drop in systemic arterial blood pressure caused by the i.v. injection of the peptides before and after the administration of carboxypeptidase B.[12] The weight of the carboxypeptidase B used refers to a purified enzyme preparation as described in a previous publication.[12]

When the fate of the injected enzyme was studied, anesthetized guinea pigs were exsanguinated 30–60 min after the injection. Tissues (e.g. kidney or liver) were perfused with cold saline to free them from blood. Wet-weighed samples of tissues were homogenized in saline in a glass homogenizer with a Teflon pestle. The homogenate was centrifuged at 14,500 *g* for 30 min. Carboxypeptidase B activity was determined in the supernatant.

Urine and blood serum samples were diluted and used without further treatment.

The effects of the bradykinin analogues on the systemic blood pressure were compared with bradykinin according to Burn *et al.*[13]

The influence of bradykinin and Lys^9-bradykinin on the isolated guinea pig ventricle strips was also studied according to the method of Sanyal and Saunders.[14] The ventricle strips were driven electrically at a frequency of 1·5/sec by a Grass S-5 stimulator delivering 60-V square wave pulses of 16-msec duration. The ventricle strips were allowed to equilibrate for 30 min before drugs were added. The bradykinin used in the experiments with the ventricle strip preparations was dissolved in saline, and the solution contained no chlorobutanol.

RESULTS

Both bradykinin and Lys^9-bradykinin transiently lower the mean systemic arterial pressure of the guinea pig. The ratio of activity of the peptides varied from animal to animal. Usually Lys^9-bradykinin was 2–3 times more hypotensivethanbradykinin when compared on the basis of weight. The mean dose of bradykinin for lowering the blood pressure by 15–30 per cent was 0·52 μg/kg, while that of Lys^9 bradykinin was 0·18. The difference was significant on the $P < 0{\cdot}05$ level (Table 1).

Surprisingly, Lys^{10}-kallidin was not significantly more active than kallidin (Table 1). In six guinea pigs, 0·64 μg kallidin/kg was equivalent to 0·48 Lys^{10}-kallidin.

Exploratory studies indicated that bradykinin was more effective by one or two orders of magnitude than Lys^9-bradykinin on the rat uterus, rabbit blood pressure, or the autoperfused dog hind leg. The latter data are in agreement with the results obtained by others.[1, 3]

In one experiment Cit^9-bradykinin (citrulline^9-bradykinin) was at least 50 times weaker than bradykinin on the guinea pig systemic blood pressure. Orn^9-bradykinin (ornithine^9-bradykinin) in two experiments was about 10 times less active than bradykinin. Both of these peptides were relatively ineffective in other tests as well.

TABLE 1. DOSES (i.v.) OF BRADYKININ, KALLIDIN, LYS^9-BRADYKININ AND LYS^{10}-KALLIDIN THAT HAVE AN EQUIVALENT EFFECT ON THE SYSTEMIC ARTERIAL BLOOD PRESSURE OF THE GUINEA PIG

No. of animals	Dose* (μg/kg)	Dose (μg/kg)
21	Bradykinin 0·52 ($\pm$0·06 S.E.)	Lys^9-bradykinin 0·18 ($\pm$0·03 S.E.)
6	Kallidin 0·64	Lys^{10}-kallidin 0·48

* Dose that lowers blood pressure 15 to 30 per cent.

Potentiation of the effects

Our previous studies showed that various compounds can potentiate *in vivo* the effects of kinins in the guinea pig.[15] These are mostly metal-complexing agents which inhibit *in vitro* the enzymatic destruction of the peptides by binding a metal cofactor. Their action *in vivo* is also attributed to blocking of the inactivation of the peptides in blood. In the present studies 14 guinea pigs were treated i.v. with 2-mercaptoethanol (66 mg/kg; range, 60–90). Two guinea pigs received 56 mg mercaptopropionic acid/kg.

In agreement with our previous findings, the effect of bradykinin and kallidin and also that of their analogues was greatly increased by pretreating the guinea pigs with the mercapto compounds. The drop in the mean systemic arterial blood pressure was increased and prolonged by the peptides administered, which include Lys^9-bradykinin, Lys^{10}-kallidin, and Orn^9-bradykinin. A typical experiment is shown in Fig. 2. Usually the duration of hypotension caused by bradykinin was more potentiated by mercaptoethanol than by the effects of its Lys^9 analogue.

On the average, mercaptoethanol increased the drop in mean arterial blood pressure which follows the injection of Lys^{10}-kallidin from 23 to 44 per cent and that of Lys^9-bradykinin from 20 to 41 per cent. Table 2 summarizes the results and shows the mean values obtained. Details of experiments with bradykinin have been published previously.[15]

After mercaptoethanol treatment, 20 per cent or 10 per cent of the original dose of Lys^9-bradykinin or Lys^{10}-kallidin respectively caused the same drop that the higher dose of the peptide had done before the inhibitor was given.

Blockade of the effects

Carboxypeptidase B of the swine pancreas inactivates bradykinin and kallidin by cleaving the C-terminal arginine of the peptides. This has been shown *in vitro*[16] and also *in vivo*[2, 12, 17, 18] in rabbits, rats, cats, dogs, and guinea pigs. In these animals i.v. injections of purified carboxypeptidase B prevented the cardiovascular action of the peptides by very rapidly inactivating them in the circulation.

Guinea pigs were injected i.v. with purified swine pancreatic carboxypeptidase B. When 5–7 mg of the enzyme/kg was given in a single injection, the drop in blood pressure which normally would follow the subsequent injection of Lys^9-bradykinin

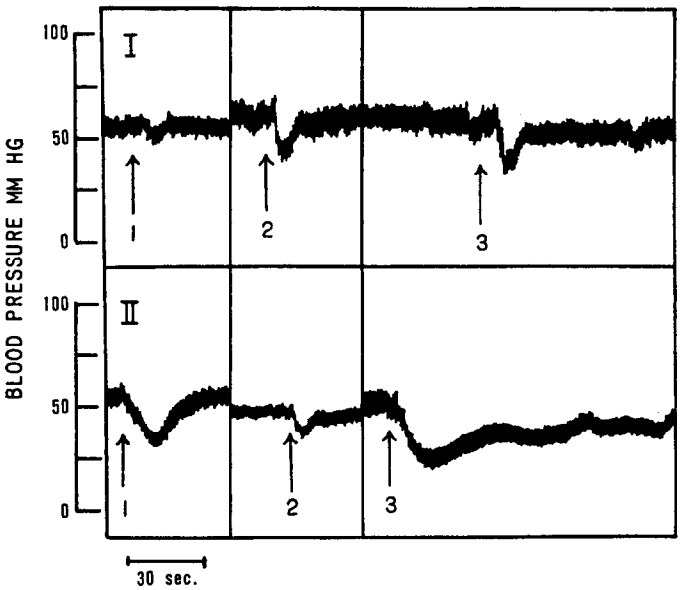

FIG. 2. Potentiation of the hypotensive effects of bradykinin and Lys^9-bradykinin with mercaptoethanol in a 0·5-kg guinea pig. Histamine effect was not increased. Dose per kg weight: (1) 0·04 μg Lys^9-bradykinin; (2) 0·55 μg histamine; (3) 0·2 μg bradykinin. Between I and II 66 mg 2-mercaptoethanol was injected i.v.

TABLE 2. POTENTIATION OF THE HYPOTENSIVE EFFECT OF LYS^9-BRADYKININ LYS^{10}-KALLIDIN BY MERCAPTOETHANOL (60–80 mg/kg i.v.) IN THE GUINEA PIG

No. of animals	Per cent drop in systemic blood pressure	
	Before	After
	Lys^9-bradykinin	
9	20	41
	Lys^{10}-kallidin	
5	23	44

was abolished in eight guinea pigs (Fig. 3) and reduced in one. The injection of carboxypeptidase B at the 3 mg/kg dose level had to be repeated once before Lys^9-bradykinin was completely blocked. In contrast, the activity of bradykinin was readily abolished by a single dose of 3 mg carboxypeptidase/kg. Two guinea pigs, which were made more sensitive to the peptides by pretreatment with mercaptopropionic acid, were protected against Lys^9-bradykinin by injection of carboxypeptidase B (5mg/kg).

Fate of injected enzyme

The level of injected carboxypeptididase in the circulating blood decreases in an exponential manner. The half-life of the enzyme in the circulation of cat was about 30 min.[12] In guinea pigs which were treated with a single injection of carboxypeptidase, the return of the full effect of bradykinin varied from 20 min to 2·5 hr.

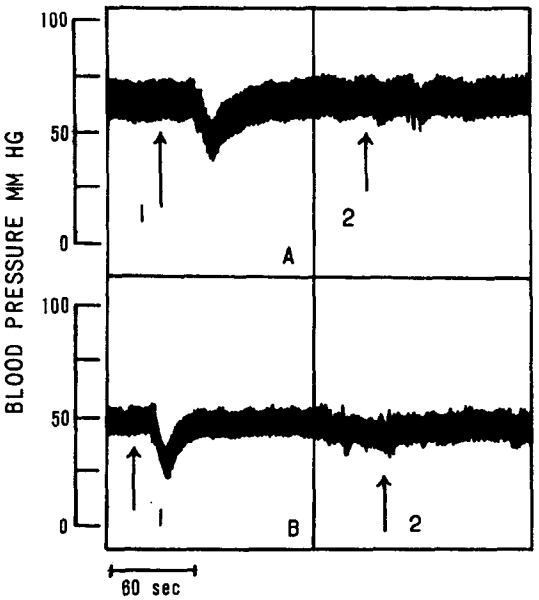

FIG. 3. Blocking the effect of Lys⁹-bradykinin with pancreatic carboxypeptidase B in two guinea pigs (A, B). Weights: A = 0·21 kg, B = 0·31 kg. Dose per kg weight: A = 0·5 μg, B = 0·64 μg. Between 1 and 2, 6·8 mg of carboxypeptidase B was injected i.v. to A and 6·7 mg to B.

The current investigation showed that after the enzyme disappears from the circulation of the guinea pig it is taken up in large amounts by the kidney. The urine also contained substantial quantities of carboxypeptidase B.

The activity of the injected enzyme in the organs, blood, and urine of 19 carboxypeptidase B-treated guinea pigs was estimated by measuring the hydrolysis of hippuryl-L-arginine.[11, 18] Highest concentrations of the enzyme were found in the kidney. The mean rate of hydrolysis of hippuryl-L-arginine in nine animals was $3{\cdot}3 \times 10^{-2}$ μmole/min per mg tissue (range, 0·7–9·4). One ml urine hydrolyzed 5·7 μmoles substrate/min (range, 0–9·9; 8 animals). Serum values varied, depending on the dose of carboxypeptidase B administered and on the time of death after the injection. Generally they were of the same order of magnitude as the values found in the urine. Brain, lung, and one bile sample showed no enzymatic activity. Of the nine liver samples tested, the activity of only three was above the untreated control ($2{-}8 \times 10^{-4}$ μmole/min/mg).

Inactivation in serum

The enzymatic inactivation of Lys⁹-bradykinin by guinea pig serum was compared

with that of bradykinin. To compensate for the difference in the activity of the peptides on the isolated rat uterus, the following system was used: 3·3 μg of each peptide/ml was incubated with guinea pig serum (1:400 dilution) in a 0·1 M Tris buffer, pH 7·4. The samples of the incubation mixture containing Lys^9-bradykinin were injected directly into the muscle bath; the bradykinin samples were diluted 200-fold first and then injected. Under the *in vitro* conditions employed, bradykinin and Lys^9-bradykinin were inactivated by guinea pig serum at approximately the same rate.

Effect on ventricle strip

Bradykinin in doses of 0·5, 5·0, and 10 μg per ml bath solution produced a slight, insignificant increase in the contractile force of the ventricle strip of guinea pig heart muscle. In two experiments the same doses of Lys^9-bradykinin had no noticeable effect.

DISCUSSION

These experiments demonstrated that the Lys^9 analogue of bradykinin is a stronger hypotensive agent in the guinea pig than is the parent compound. As observed with bradykinin, the hemodynamic activity of Lys^9-bradykinin was potentiated by mercaptoethanol and blocked by carboxypeptidase B.

The stronger activity of Lys^9-bradykinin in the guinea pig is puzzling, since this peptide is much less active than bradykinin in another test (e.g. guinea pig ileum) or in other animals (Table 3). Lys^{10}-kallidin, however, was not more potent in the

TABLE 3. BIOLOGICAL EFFECTS OF LYS^9-BRADYKININ AND LYS^{10}-KALLIDIN

	Rat uterus*	Guinea pig* ileum	Rabbit blood* pressure	Rat blood* pressure	Guinea pig blood pressure
Bradykinin	1	1	1	1	1
Lys^9-bradykinin	1/200–1/500	1/5	1/10–1/30	1/25	2·9
Kallidin	1/10–2/3	1/3	2	3·3	2/3
Lys^{10}-kallidin	1/500	1/100	1·5		2/3

* Refs. 1–3 and Erdös, unpublished.

present study than kallidin. Orn^9- and Cit^9-bradykinin were fairly ineffective in a few experiments. Of the peptide analogues in which the carboxyl-terminal arginine was replaced with another basic amino acid, only the Lys^9 derivative was more potent than bradykinin.

The activity of Lys^9-bradykinin was abolished by pretreating guinea pigs with i.v. injection of purified pancreatic carboxypeptidase. The blocking *in vivo* of the effects of bradykinin rests upon the very rapid hydrolysis of the peptide at the carboxyl-terminal end. The mean dose of carboxypeptidase B used in these experiments was larger than the amount of the enzyme necessary to block the effect of bradykinin.[12] This finding is in agreement with the reported properties of carboxypeptidase B. Swine pancreatic carboxypeptidase B hydrolyzes hippuryl-L-arginine faster than hippuryl-L-lysine at the 1×10^{-3} M concentration level.[11]

Injected carboxypeptidase B was removed from the circulation by the kidney and excreted in the urine in active form. Essentially similar results were obtained in cats,

dogs, and rats, although the liver of some of these animals contained more of the injected enzyme than guinea pigs (Erdös, unpublished). In dogs, injected carboxypeptidase B appeared first in the hind limb lymph after a systemic infusion,[18] when their renal arteries were occluded.

2-Mercaptoethanol and mercaptopropionic acid strongly potentiated the systemic hypotension caused by Lys^9-bradykinin. These and other agents increase the effects of bradykinin also in the guinea pig.[15] Some of them are effective in other species as well.[2] These compounds can complex metals and they inhibit *in vitro* the enzymatic hydrolysis of bradykinin in human and animal blood. It has been assumed that their action *in vivo* rests also on the inhibition of the enzymatic inactivation of bradykinin in blood.[19]

Diluted guinea pig serum inactivated bradykinin and Lys^9-bradykinin equally fast. Thus a decreased enzymatic metabolism could not explain the stronger hypotensive activity of this peptide analogue of bradykinin.

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