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INHIBITION *IN VIVO* OF THE ENZYMATIC INACTIVATION OF BRADYKININ AND KALLIDIN*

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Abstract—Nine inhibitors of the metabolism of bradykinin effective *in vivo* in the guinea pig were described. Some were also tested and found active against kallidin and 6-glycine bradykinin *in vivo* or against hippuryl-L-lysine and bradykinin *in vitro*. The compounds potentiated the hypotensive effect of the kinins, presumably by inhibiting the enzymatic inactivation in blood. Results of these experiments are in good agreement with the conclusions of *in vitro* studies.

THE DIFFERENCE in the structure of bradykinin and kallidin is the additional N-terminal lysine in the decapeptide kallidin.[1, 2] The two peptides, however, have the same C-terminal arginine, which is susceptible to attack by carboxypeptidases. This has been shown *in vitro*,[3] and also by experiments *in vivo*,[4, 5] where pretreatment of various laboratory animals with purified pancreatic carboxypeptidase B abolished the effect of the peptides. Our previous communications attributed the inactivation of bradykinin and probably kallidin on treatment with human plasma fraction IV-1 to a metalloenzyme named carboxypeptidase N.[6, 7] (This enzyme can be distinguished from carboxypeptidase B, which occurs in zymogen form in the pancreas.[4, 7–9]) The destruction of the activity of bradykinin in the sera of some animals resembles that in human plasma.[4] The inactivation of kallidin is complicated by the fact that its N-terminal lysine–arginine bond is cleaved in human plasma by another enzyme, presumably an aminopeptidase.[4, 10] Thus, preceding the hydrolysis of the C-terminal arginine bond, kallidin can be partially converted[4] or, according to others,[10] fully transformed to bradykinin.

The present investigation extends the *in vitro* studies on these enzymes by exploring the inhibition of the inactivation of bradykinin and kallidin *in vivo* in the guinea pig. A preliminary report on this subject was published recently.[11]

MATERIALS AND METHODS

Synthetic bradykinin and synthetic, but only partially pure, kallidin were used in these experiments. The effect of the peptides on the blood pressure and respiration of guinea pigs, weighing 250 to 400 g, in pentobarbital narcosis was registered with a Grass polygraph. Bradykinin or kallidin was injected into the jugular vein at 15-min intervals. After selecting a dose of the peptide which lowered the blood pressure only slightly the inhibitor was given i.v., then the injections of the selected dose of bradykinin or kallidin were repeated at the same 15-min intervals. The inhibition of the

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enzymatic destruction of bradykinin or kallidin was evaluated by observing the increased and more persistent hypotension caused by the peptide after the injection of the inhibitors. In control studies, histamine, norepinephrine, or eledoisin replaced the peptides. In all, 92 guinea pigs were used. When the effect of the inhibitor had disappeared the animal was usually tested again. The use of some inhibitors was restricted by their poor water solubility or toxic side effects.

The inhibition *in vitro* of the hydrolysis of bradykinin in human serum was studied by using a previously described technique with the isolated, surviving rat uterus.[7, 12] Serum contains a carboxypeptidase which is similar to or identical with carboxypeptidase N and hydrolyzes hippuryl-L-lysine.[4, 13, 14] The activity of this serum enzyme was assayed in a Cary model 15 recording UV spectrophotometer at wavelength 2540Å at 37° in a 0·1 M Tris buffer of pH 7·4.[9, 14] The source of enzyme was diluted human serum (1:40 v/v). Swine pancreatic carboxypeptidase B was obtained from Worthington Biochemical Corp., or purified in this laboratory.

RESULTS

Experiments in vivo

Table 1 summarizes the results with bradykinin and Table 2 with kallidin. Only small quantities of kallidin were available, hence fewer experiments were done with this

TABLE 1. SUMMARY OF RESULTS WITH BRADYKININ

	Inhibitor	No. of experiments	Dose of inhibitor (mg/kg)	Dose of bradykinin (μg/kg)	Drop in mean blood pressure (%) Before*	After*
I	2-Mercaptoethanol	15	67 (45–83)†	1·0 (0·03–2·0)	10 (0–24)	42 (20–55)
II	β-Mercaptoethylamine	11	90 (50–165)	0·8 (0·4–1·2)	10 (0–20)	16 (7–37)
III	3-Mercaptopropionic acid	13	41 (25–74)	1·1 (0·5–1·8)	13 (0–25)	44 (33–57)
IV	α-Thioglycerol	13	242 (108–429)	0·6 (0·3–1·7)	11 (0–26)	32 (20–52)
V	2,3-Dimercaptopropanol (BAL)	15	16 (5–31)	0·9 (0·3–1·8)	13 (0–28)	48 (31–58)
VI	Diethyldithiocarbamic acid	13	71 (25–100)	1·2 (0·3–2·0)	13 (0–20)	38 (22–58)
VII	L-Penicillamine	13	130 (37–200)	1·2 (0·4–2·2)	11 (6–23)	23 (12–41)
VIII	8-Hydroxy-5-quinoline sulfonic acid	9	126 (50–165)	0·5 (0·3–0·6)	5 (0–13)	31 (23–42)
IX	Ca-EDTA	8	175 (162–198)	0·9 (0·5–1·2)	8 (7–17)	27 (18–33)
X	Glutathione	4	88 (66–111)	1·2 (1·1–1·2)	10 (0–19)	24 (20–30)

* Injection of inhibitor.
† Range.

peptide. The most active compounds were I (2-mercaptoethanol) (Fig. 1); III (3-mercaptopropionic acid) (Fig. 2); and V (2,3-dimercaptopropanol [BAL]) (Fig. 3) These agents had a relatively long-lasting activity; frequently the hypotension caused by bradykinin or kallidin was potentiated for several hours. After the first rapid drop

TABLE 2. SUMMARY OF RESULTS WITH KALLIDIN

Inhibitor	No. of experiments	Dose of inhibitor (mg/kg)	Dose of kallidin (μg/kg)	Drop in mean blood pressure (%) Before*	After*
2-Mercaptoethanol	5	60 (55–69)†	2·3 (0·7–4·0)	6 (0–14)	34 (23–47)
α-Thioglycerol	2	189 (163–215)	1·0 (0·9–1·1)	16 (12–20)	50 (48–52)
2,3-Dimercaptopropanol (BAL)	11	13 (2–25)	2·2 (1·7–3·0)	13 (0–24)	38 (20–53)

* Injection of inhibitor.
† Range.

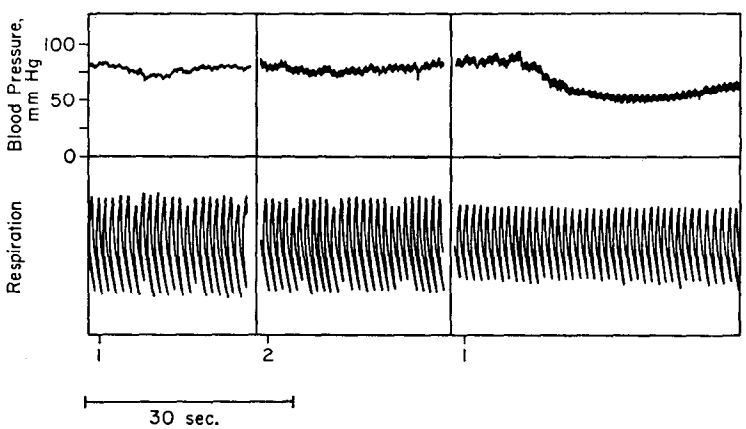

FIG. 1. Potentiation of the effect of kallidin by mercaptoethanol (compound I). Male guinea pig, 0·36 kg. Last injection of kallidin shown was given 35 min after 2. Dose per kg weight: 1, 3·5 μg kallidin; 2, 55 mg mercaptoethanol.

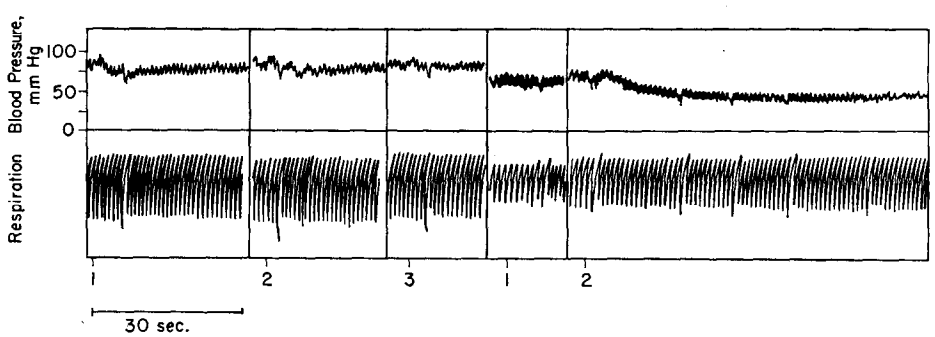

FIG. 2. Potentiation of the effect of bradykinin by mercaptopropionic acid (compound III). Male guinea pig, 0·4 kg. Time between first and last injection shown: 53 min. Dose per kg weight: 1, 0·8 μg histamine; 2, 1 μg bradykinin; 3, 57 mg mercaptopropionic acid.

that followed injection of the peptide, the blood pressure returned to the preinjection level much more slowly in the animals treated with inhibitor. Compound III became more effective 20 to 35 min after the administration than immediately after it. Compound V is the most potent one, but it is relatively poorly water soluble and quite toxic in that it lowers the blood pressure and affects the respiration of the guinea pigs unfavorably (Fig. 3). Compounds I, III, and IV (α-thioglycerol) were well tolerated, but

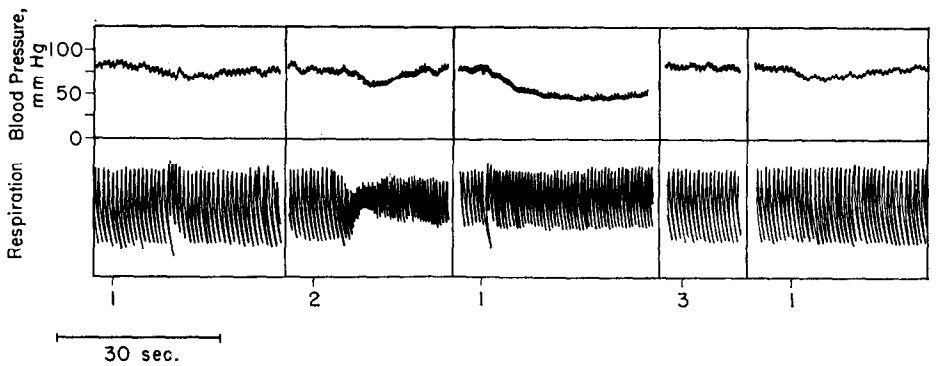

FIG. 3. Demonstration that carboxypeptidase B abolishes the potentiating effect of BAL (compound V) on kallidin. Male guinea pig, 0·44 kg. Dose per kg weight: 1, 2·5 μg kallidin; 2, 25 mg BAL; 3, 1·6 mg carboxypeptidase B (104 Worthington units/mg). Kallidin was injected at 15-min intervals.

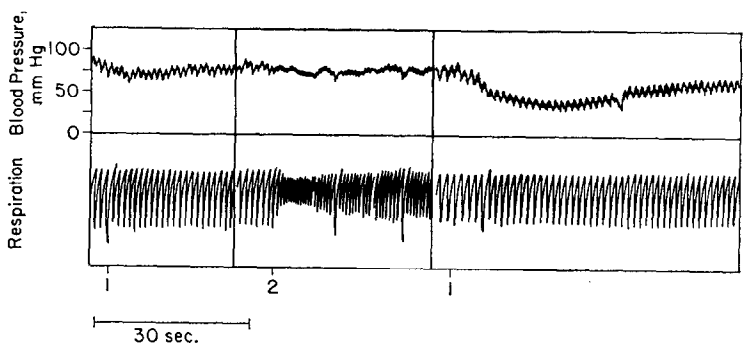

FIG. 4. Potentiation of the effect of bradykinin by diethyldithiocarbamic acid (compound VI). Male guinea pig, 0·35 kg. Dose per kg weight: 1, 1 μg bradykinin; 2, 75 mg diethyldithiocarbamic acid.

VI (diethyldithiocarbamic acid) had side effects (Fig. 4). The potentiation by II (β-mercaptoethylamine) was negligible and VII (L-penicillamine) was only slightly active. Despite large doses given, the effect of VIII (8-hydroxy-5-quinoline sulfonic acid) (Fig. 5) and IX (Ca-EDTA) disappeared within half an hour. Compound X (glutathione) also increased the effect of bradykinin (Table 1), and in two experiments it increased the effects of kallidin and in three, of 6-glycine bradykinin (not shown).

The increased sensitivity to bradykinin and kallidin in the guinea pig pretreated with inhibitors IV or V (Fig. 3) was abolished by an injection i.v. of purified carboxypeptidase B.

Compound IV also potentiated the 6-glycine analogue of bradykinin; the results with this peptide are included in Table 1 under bradykinin. Inhibitor III did not influence significantly the effect of histamine in control experiments (Fig. 2). The following compounds gave negative results in exploratory studies: mercaptosuccinic acid (19 to 150 mg/kg); Co-EDTA (194 to 231 mg/kg); ϵ-amino-n-caproic acid (111 to 135 mg/kg); benzoylarginine (27 to 57 mg/kg); cysteine (37 mg/kg); and 8-hydroxyquinoline sulfate (25 to 83 mg/kg). The last agent was, however, very toxic. Compound I did not affect the rise in blood pressure caused by 0·3 μg norepinephrine/kg, or the fall caused by the i.v. injection of the hypotensive peptide eledoisin (0·2 μg/kg).

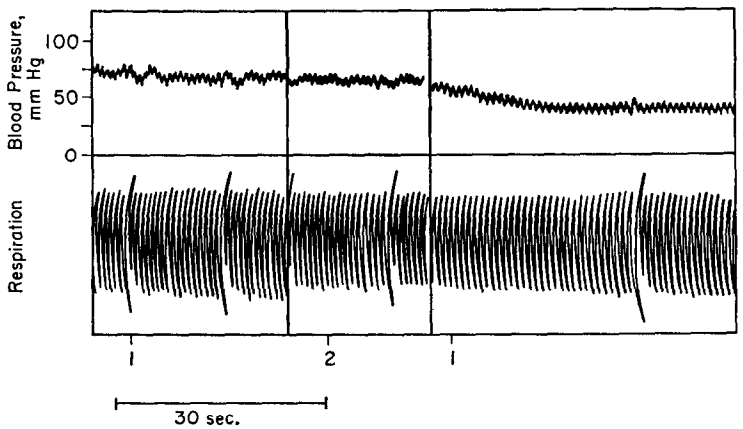

FIG. 5. Potentiation of the effect of bradykinin by 8-hydroxy-5-quinoline sulfonic acid (compound VIII). Male guinea pig, 0·4 kg. Dose per kg weight: 1, 0·4 μg bradykinin; 2, 125 mg 8-hydroxy-5-quinoline sulfonic acid.

Experiments in vitro

Compounds I ($1 \cdot 10^{-3}$ M) and V ($1 \cdot 10^{-4}$ M) completely inhibited the hydrolysis *in vitro* of hippuryl-L-lysine in diluted human serum, when measured in the spectrophotometer. Compound IX was shown previously to inhibit the destruction of bradykinin in diluted human plasma;[4, 6, 7] V and VIII caused the same effect in rat plasma.[15, 16] In the present studies the inactivation of bradykinin in diluted human serum was measured on the isolated rat uterus, and this inactivation was inhibited partially by benzoylarginine ($3 \cdot 10^{-3}$ M) and to a greater extent by VIII ($3 \cdot 10^{-4}$ M) and 8-hydroxyquinoline sulfate ($3 \cdot 10^{-4}$ M).

DISCUSSION

The success with compounds I, and III–X *in vivo* supports the conclusions of our previous studies *in vitro*.[4, 6, 7, 17] Accordingly, the action of these inhibitors in the guinea pig rests on binding of a metal cofactor of the enzyme, presumably a carboxypeptidase, that inactivates kinins. The assumption that the compounds tested increased the effect of bradykinin and kallidin by preventing their metabolism in the blood is corroborated by the following considerations. Among laboratory animals, guinea pig serum is very effective in inactivating kinins;[18] the short duration of action of the

peptides in this species may be attributed to that fact. The inhibitors used here, although varying greatly in structure, are chelating agents, and many of them inhibit metalloenzymes *in vitro*, such as carboxypeptidase A or alcohol dehydrogenase.[19]

Several of the inhibitors were tested and found effective in inhibiting carboxypeptidases also in our *in vitro* experiments. Other chelating agents were shown previously to inhibit the destruction *in vitro* of bradykinin in various sera.[4, 6, 7] Of the —SH agents, cysteine has been used extensively,[20] although the mechanism of action has been only recently elaborated.[6, 7, 21] Cysteine also potentiated kallidin in experiments[20] in dogs *in vivo*, but it was inactive in our hands in guinea pigs. The species difference may explain this discrepancy. After pretreatment of the guinea pig with an inhibitor, the increased response to bradykinin or kallidin was abolished by injecting purified carboxypeptidase B. (This enzyme can inactivate these kinins by cleaving their C-terminal arginine, but does not affect other hypotensive agents.[4])

Although the thio-compounds are quite unstable in solution, they were the most active inhibitors found. Those with a free alcohol or carboxyl group were more active than amines. Of the EDTA derivatives, Co-EDTA complex was inactive whereas Ca-EDTA inhibited. This can be explained by the fact that cobalt has a much higher affinity for EDTA than has calcium; thus the inhibition by Ca-EDTA is probably due to the exchange of the alkaline earth[22] in the complex for a metal with a higher stability constant—e.g. cobalt.

The inhibition of the destruction of kallidin might be complicated by the fact that the enzyme that can cleave the N-terminal lysine of kallidin is probably an aminopeptidase,[4, 10] and is thus also sensitive to sequestering agents.

Eledoisin, the hypotensive peptide, does not have a free carboxyl group; it is therefore resistant to carboxypeptidase.[23] In contrast to kinins, the effect of this peptide was not potentiated by inhibitor I, which is in good agreement with our other findings.

As a result of our characterization of the enzymatic inactivation of kinins,[6, 17] Rocha e Silva[16] has suggested the use *in vivo* of a sequestering agent (V) for preserving the action of bradykinin.

The enzymatic inhibition *in vivo* is influenced not only by the affinity of the sequestering agent for the metal ion, but perhaps by other factors such as the rapidity of equilibration between the metal and the complexing agent, the distribution and metabolism of the inhibitor in the body, and the reversibility of inhibition.

The inhibition of the enzymatic destruction of bradykinin and kallidin may help in studies of the release or fate of these peptides in the guinea pig, where their short half-life causes considerable difficulty.

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