Cardiovascular and Gastrointestinal Effects of Bradykinin and Its Potentiation by Thiols in Rats

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Abstract □ Cysteine, 2-propanethiol, and 2-mercaptoethylamine were found to potentiate the action of bradykinin on the rat blood pressure. These same thiols were ineffective on the *in vitro* rat ileum, although on the guinea pig ileum only 2-propanethiol was observed to be ineffective. The potentiation is presumed to result from thiol complexation of the zinc moiety in the active center of the carboxypeptidase B-type enzyme (responsible for the lysis of bradykinin), hence allowing the nonapeptide to exert a greater effect. Thus the results tend to support the hypothesis that this enzyme may play an important role in terminating the action of bradykinin on rat blood pressure and guinea pig ileum, but in the rat ileum other enzyme systems may be involved.

Keyphrases □ Bradykinin—gastrointestinal, cardiovascular effects □ Blood pressure—bradykinin effect □ Intestinal segments—bradykinin effect □ Thiols—bradykinin activity potentiation

Bradykinin possesses a potent stimulating action upon the *in vitro* guinea pig ileum, rabbit intestine, rat uterus, stomach and ileum, a relaxant action on the rat duodenum and colon (1–4), and produces a pronounced fall in blood pressure in the rabbit, cat, rat, and guinea pig (5–7). The preadministration of carboxypeptidase B has been found to inactivate, and hence block effectively, many of these *in vivo* and *in vitro* actions of bradykinin (8, 9). Additionally, Erdos *et al.* discovered an enzyme in human plasma fraction IV-1 that also was capable of inactivating bradykinin. This enzyme has many of the characteristics of carboxypeptidase B, and though discernible from it, was termed carboxypeptidase N (9, 10).

Several investigations have shown that the effects of bradykinin on guinea pig ileum, blood pressure, and respiration, and rat uterus may be potentiated by a variety of agents *i.e.*, 2,3-dimercaptopropanol (BAL), EDTA (Versene), thioglycolic acid, cysteine, 8-hydroxyquinoline, 2-mercaptoethanol, and 2-mercaptoethylamine. These investigations showed a consistant potentiation of the normal bradykinin effect (save one: 8-hydroxyquinoline) on those tissues and systems studied (11–13). It is noteworthy that the common property possessed by the aforementioned compounds is their ability to complex divalent metals. The removal of the zinc moiety in the active center of carboxypeptidase or its complexation leads to the inactivation of the enzyme (14–16). Consequently, the inactivation of carboxypeptidase B-type enzymes with sequestering agents allows bradykinin to exert an even greater effect.

The purpose of the present investigation was primarily to discern whether the cardiovascular and *in vitro* gastrointestinal responses of the male albino rat to bradykinin were both qualitatively and quantitatively similar to those of the guinea pig and secondarily to observe if thiol inactivation of carboxypeptidase is modified by the presence of additional functional groups.

EXPERIMENTAL PROCEDURE

In Vivo Blood Pressure—Healthy male rats of the Sprague-Dawley (Huntington Farms) variety were used in all experiments. The rats weighed approximately 350 g. (285–400 g.) and were housed in the animal house at least 24 hr. prior to testing. It was decided to utilize a dose of thiol test compound that, by itself, had no observable effect on the blood pressure when administered alone; in this way control procedures were simplified. The volume of the test drug never exceeded 0.25 ml. and was never less than 0.20 ml. With the administration of a dose any larger than the determined maximum dose, a slight and transitory depression of the blood pressure was observed. All thiol test compounds were made to a concentration of 4×10^{-3} mole/l. in distilled water, and only one thiol was used in any one animal.

The only exception to the above procedure was in the case of cystine. By virtue of its low solubility in physiologic pH ranges, it was necessary to dissolve this amino acid in solvents below pH 2 or above pH 11. This was done by weighing an amount of cystine that, with proper dilution, would yield a solution of 2×10^{-3} mole/l. The diluting solution was either 0.1 *N* HCl or 0.1 *N* NaOH; as soon as pH 2 (or 11) was reached, it was brought to volume with distilled water. Glycine, in a concentration of 4×10^{-3} mole/l. adjusted to the above pH values, was utilized, rather than acid or base alone as solvent controls, in order to mimic the conditions present in the cystine solutions.

The rats were anesthetized by intraperitoneal administration of a 50% urethane solution, in a dose of 125 mg./100 g. body weight.

All test compounds were administered *via* the right jugular vein, which was cannulated with tubing (Clay Adams PE 60); all blood pressures were taken from the left carotid artery, cannulated with tubing (Clay Adams PE 40) filled with heparinized saline, connected to a pressure transducer (E & M P-1000), and recorded on a physiograph (E & M Physiograph "Four").

The bradykinin was supplied[1] as synthetic bradykinin SBR-640, in 1.0-ml. ampule, each containing 0.1 mg. of the active polypeptide. This was diluted with distilled water prior to use so that 1.0 ml. contained 0.2 mcg. The doses utilized were of the magnitude of 0.05–0.15 mcg. A series of doses was used in this range, none of which would elicit a maximum response. All drugs were washed through the cannula with minimal amounts of normal saline.

The rat's blood pressure was allowed to equilibrate for approximately 30 min. prior to the infusion of drugs. The decrease in mean blood pressure, after the infusion of a control dose of bradykinin, was calculated as a percentage of the base line mean blood pressure. Subsequently, the pressure was allowed to re-equilbrate (about 3 min.) and the predetermined dose of thiol was infused, followed immediately by the same control dose of bradykinin. If increased depression of the mean blood pressure occurred, a larger dose of bradykinin was chosen to mimic the depression of the thiol-potentiated decrease.

In Vitro Ileum—Male Sprague-Dawley (Huntington Farms) rats also were utilized in this part of the experiment, and divided into two groups weighing approximately 300 g. (285–360 g.) and 175 g. (150–185 g.). These were further subdivided into groups that were nonfasted and those that were fasted for 24 hr. The animals were sacrificed by cervical dislocation followed quickly by the removal of three portions of ileum, each about 2 cm. in length. The test segment was suspended in a 20-ml. tissue bath with air-bubbled,

[1] Sandoz Pharmaceutical, Hanover, N. J.

Table I—Compounds Used and Functional Groups Present

Test Agent	Functional Groups Present —SH	NH_2—	—COOH
2-Propanethiol[a]	X	—	—
2-Mercaptoethylamine[b]	X	X	—
Cysteine[c]	X	X	X
Cystine[c]	—	X	X
Controls			
Methionine[c]	—	X	X
Glycine[c]	—	X	X

[a] Aldrich Chemical Co., Milwaukee, Wis. [b] Dow Chemical Co., Midland, Mich. [c] Sigma Chemical Co., St. Louis, Mo. All the free base.

Table II—Comparison of Potentiation of Bradykinin by Various Test Agents on the Blood Pressure of the Male Rat

Test Compd.	No. Experiments	Mean Percent Difference[a]	SEM ±[b]	p Value[c]
2-Mercaptoethylamine	14	7.03	1.012	0.001
Cysteine	24	5.90	0.779	0.001
2-Propanethiol	10	8.61	1.546	0.001
Methionine	20	0.26	0.600	0.700[d]

[a] Mean percent difference in depressor response. [b] Standard error of the mean. [c] As computed from paired, *t* test (20). [d] Not significant.

atropinized (1 mg./l.) deJalon solution, which was maintained at 31 ± 1°. Those segments that were not used immediately were stored in air-bubbled, atropinized deJalon solution at 4°, for a maximum of up to 2 hr.

The test segments were allowed to equilibrate for at least 15 min. prior to experimentation. After each sequence, the bath was drained and filled twice, with approximately 5 min. elapsing until the next run. No two thiol compounds were used on any one segment. The segments were arranged so that longitudinal muscular contractions were recorded *via* connection to a microdisplacement transducer (E & M F-50).

The same thiol test agents were used as in the *in vivo* portion of the experiment. They were made to a sufficiently high concentration so that by addition of 0.25 ml., a final concentration of 2×10^{-3} mole/l. was reached in the 20-ml. bath. The same SBR-640 synthetic bradykinin was also used, but it was diluted so that 1.0 ml. equaled 2.0 mcg. of polypeptide.

Small doses of bradykinin, usually 0.5 mcg., were added to the bath and the response measured after a contact time of 90 sec. The bath was drained, washed twice, and about 5 min. were allowed to elapse until the next run. The process was repeated several times in order to obtain an average response for that dose of bradykinin. Any tissue segment that did not give consistent responses to several consecutive doses of bradykinin was considered unsatisfactory and discarded. After this base line was obtained, the 0.25 ml. of the thiol compound was added to the bath and the same dose of bradykinin was added 30 sec. later. If the thiol caused a potentiation, an attempt was made to ascertain how much more bradykinin would be needed to mimic the thiol-potentiated dose. The test agents selected and functional groups present are shown in Table I.

In addition to their usefulness in possessing varying functional groups, they were soluble (except for cystine), relatively stable, had similar molecular weights, and were nontoxic to the animal in the dose administered.

RESULTS

The depressor effect of bradykinin on the rat blood pressure was potentiated by 2-mercaptoethylamine to a significant degree (Tables

Table III—Comparison of Mimicking Dose of Bradykinin to Potentiated Bradykinin on the Blood Pressure of the Male Rat

Test Compd.	No. Experiments	Percent Increase over Control	Mean Percent Difference[a]	SEM ±[b]	p Value[c]
2-Mercaptoethylamine	14	50	0.350	0.228	0.20[d]
Cysteine	11	25	1.809	0.678	0.05
	6	40	0.483	0.665	0.60[d]
	7	50	2.057	0.643	0.02
2-Propanethiol	10	50	0.430	0.271	0.20[d]

[a] Mean percent difference in depressor response. [b] Standard error of the mean. [c] As computed from paired *t* test (20). [d] Not significant.

Table IV—Potentiation of Bradykinin by 2-Mercaptoethylamine on the Blood Pressure of the Male Rat

Rat No.	Bradykinin Control before Test Agent, mcg.	Pressure[a] Change mm. Hg	Pressure[a] Change % [b]	Test Agent, ml.	Test Agent with Bradykinin Control Pressure Change mm. Hg	Test Agent with Bradykinin Control Pressure Change %	Mimicking Dose of Bradykinin, mcg.	Pressure Change mm. Hg	Pressure Change %
1	0.100	105- 85	19.1	0.25	108- 80	25.9	0.150	115- 85	26.1
		115- 90	21.7		116- 85	26.7		120- 86	28.3
		110- 87	20.9		115- 80	30.4		115- 80	30.4
2	0.100	120-100	16.7	0.25	125- 92	26.4	0.150	128- 95	25.8
		138-120	13.1		140-114	18.6		140-115	17.9
		132-115	12.9		125- 87	30.4		122- 85	30.3
3	0.050	104- 98	5.8	0.20	103- 95	7.8	0.075	104- 96	7.7
		104- 99	4.8		105- 96	8.6		104- 95	8.7
		104- 99	4.8		103- 95	7.8		107- 99	7.5
4	0.100	130-120	7.7	0.20	130-113	13.1	0.150	128-112	12.5
5	0.100	105- 90	14.3	0.20	108- 83	23.2	0.150	110- 83	24.6
6	0.100	94- 80	14.9	0.25	102- 76	25.5	0.150	105- 77	26.7
		92- 80	13.1		95- 77	18.9		102- 81	20.6
	0.050	110-100	9.1		108- 92	14.8	0.075	114- 96	15.8

[a] Change in mean blood pressure. [b] Percent decrease in mean blood pressure.

Table V—Potentiation of Bradykinin by Cysteine on the Blood Pressure of the Male Rat

Rat No.	Bradykinin Control before Test Agent, mcg.	—Pressure[a] Change— mm. Hg	% [b]	Test Agent, ml.	Test Agent with Bradykinin Control Pressure Change mm. Hg	%	Mimicking Dose of Bradykinin, mcg.	—Pressure Change— mm. Hg	%
1	0.100	90– 75	16.7	0.20	98– 77	21.4	0.150	92– 68	26.1
		120–105	12.5		125– 98	21.6		118– 90	23.7
		125–104	16.8		128–104	18.8		129–104	19.4
		130–116	10.8		134–110	17.9		135–110	18.5
2	0.100	92– 76	17.4	0.20	90– 72	20.0	0.150	93– 72	22.6
		82– 70	14.6		88– 72	18.2		87– 71	18.4
		100– 86	14.0		94– 79	16.0		97– 78	19.6
3	0.100	118–105	11.0	0.25	117– 94	19.7	0.125	114– 97	14.9
		112– 95	15.2		99– 82	17.2		99– 82	17.2
		112–101	9.8		105– 86	18.1		83– 70	15.7
4	0.100	107– 95	11.2	0.20	100– 86	14.0	0.125	104– 91	12.5
		112–100	10.7		115– 97	15.7		113– 98	13.3
		107– 96	10.3		105– 89	15.2		109– 93	14.7
		120–100	16.7		120– 95	20.8		124– 95	23.4
5	0.100	112–105	6.3	0.25	110– 98	10.9	0.125	107– 97	9.4
		110–104	5.5		108– 97	10.2		107– 97	9.4
		110– 80	27.3		110– 72	34.6		110– 78	29.1
		100– 90	10.0		108– 88	18.5		104– 88	15.4
6	0.100	95– 81	14.7	0.20	90– 74	17.8	0.140	94– 78	17.0
		91– 85	6.6		92– 78	15.2		92– 77	16.3
		93– 79	15.1		95– 79	16.9		92– 77	16.3
7	0.100	120– 95	20.8	0.20	100– 80	20.0	0.140	102– 78	23.5
		113– 97	14.2		105– 74	29.5		110– 77	30.0
		114– 95	16.7		113– 81	28.3		112– 81	27.7

[a] Change in mean blood pressure. [b] Percent decrease in mean blood pressure.

II and IV). It was found that the thiol-potentiated value could be mimicked by a dose of bradykinin approximately 50% greater than that of the control dose (Tables III and IV).

Cysteine was also able to potentiate the bradykinin depressor effect (Tables II and V), but it was found that only about a 40% increase in bradykinin over the control dose was necessary to mimic this thiol-potentiated response (Tables III and V).

The vasodilator potential of bradykinin was also potentiated by 2-propanethiol (Tables II and VI) and about a 50% increase in bradykinin over its control values was necessary to mimic the potentiated response (Tables III, VI, and Fig. 1).

In 20 experiments performed on six rats, methionine (Table VII) was shown to exert no significant potentiating effect on bradykinin's depressor action on the rat blood pressure (Table II).

Unfortunately, the cystine and control glycine solutions gave sizeable depressions of the blood pressure, making it impossible to distinguish between their action and the drop caused by the bradykinin. In an attempt to negate these apparent pH effects, the solutions were infused several seconds (5–60 sec.) prior to the administration of the bradykinin. This modification was not successful, and the use of cystine was abandoned.

Bradykinin was able to elicit a consistent stimulatory response on the rat ileum. However, none of the thiol compounds that were able to potentiate the effect of bradykinin on the rat blood pressure were capable of potentiating the stimulatory action of bradykinin on the ileum. Rats of various weights were tried and the effects of fasting and nonfasting were also studied. These variables did not alter the effect of the thiols that were observed to be active in the *in vivo* part of this investigation (Fig. 2, bottom).

To re-evaluate the authors' method, the procedure of Cirstea was used on the guinea pig ileum (13). This investigator had been previously successful in potentiating the stimulant action of bradykinin with various thiol compounds, including cysteine, in that animal. Fasted albino guinea pigs weighing approximately 300 g. were used. These animals were sacrificed by cervical dislocation and several pieces of ileum were removed, each 2–3 cm. in length. The strips were suspended in the tissue bath in atropinized (0.1 mg./l.) Tyrode's solution and allowed to equilibrate. The temperature of the bath was 35 ± 1°. The same time sequence was followed for drug addition as utilized in the rat ileum experiments.

It can be observed (Fig. 2, top) that 2-mercaptoethylamine was able to potentiate the action of the bradykinin on the guinea pig ileum. A similar response was obtained with cysteine. Interestingly, 2-propanethiol did not potentiate the action of bradykinin on the

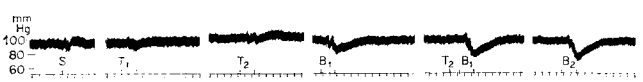

Figure 1—*Effect of 2-propanethiol and bradykinin on rat blood pressure. Key: S = saline, 0.25 ml. T_1 = 2-propanethiol, 0.25 ml. (76 mcg.); T_2 = 2-propanethiol, 0.20 ml. (60.8 mcg.); B_1 = bradykinin, 0.10 mcg.; B_2 = bradykinin, 0.15 mcg.; time interval = 5 sec.; interval between drug administration = 3 min.*

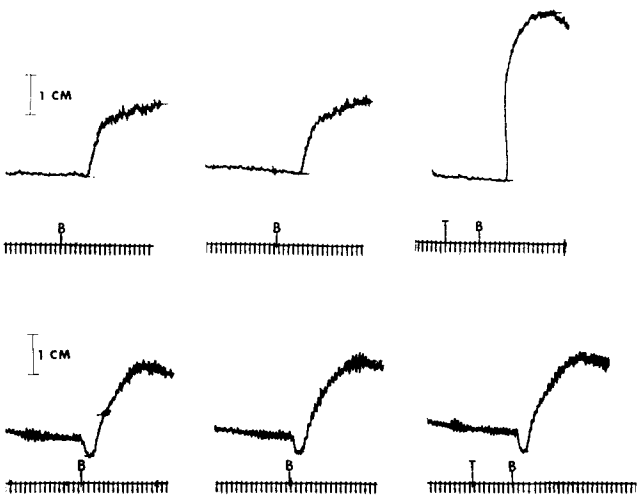

Figure 2—*Top: effect of bradykinin and 2-mercaptoethylamine on guinea pig ileum. Bottom: effect of bradykinin and 2-mercaptoethylamine on rat ileum. Key: B = bradykinin, 0.5 mcg.; T = 2-mercaptoethylamine, 0.25 ml. (3.08 mcg.); time interval = 5 sec.; interval between drug administration = 5 min.*

Table VI—Potentiation of Bradykinin by 2-Propanethiol on the Blood Pressure of the Male Rat

Rat No.	Bradykinin Control before Test Agent, mcg.	Pressure[a] Change mm. Hg	Pressure[a] Change % [b]	Test Agent, ml.	Test Agent with Bradykinin Control Pressure Change mm. Hg	Test Agent with Bradykinin Control Pressure Change %	Mimicking Dose of Bradykinin, mcg.	Pressure Change mm. Hg	Pressure Change %
1	0.100	103– 85	17.5	0.20	102– 75	26.5	0.150	105– 77	26.7
2	0.100	112– 97	13.4	0.20	112– 79	29.5	0.150	120– 85	29.2
3	0.100	95– 85	10.5	0.20	94– 68	27.7	0.150	98– 70	28.6
		107– 89	16.8		112– 78	30.4		110– 74	31.7
4	0.050	102– 92	9.8	0.20	105– 89	15.2	0.075	102– 86	15.7
		96– 86	10.4		127–108	15.0		132–114	13.6
5	0.100	100– 91	9.0	0.20	105– 92	12.4	0.150	108– 93	13.9
		109–100	8.3		113– 97	14.2		112– 95	15.2
6	0.100	100– 86	14.0	0.20	102– 79	22.6	0.150	99– 76	23.2
		102– 88	13.7		98– 80	18.4		98– 80	18.4

[a] Change in mean blood pressure. [b] Percent decrease in mean blood pressure.

guinea pig ileum, even though it was active in the rat blood pressure experiments.

DISCUSSION

The results obtained on the rat blood pressure are qualitatively similar to those of other investigators who potentiated the effect of bradykinin on the guinea pig blood pressure with thiol compounds (12, 13). The response of the guinea pig ileum also tended to confirm the observations of Cirstea, Rocha e Silva, and Ferreira (11, 13). This potentiation could well be attributed to the inactivation of a metallo-enzyme responsible for the destruction of bradykinin; however, if this is true, then it is unlikely that the same mechanism of inactivation exists in the *in vitro* Sprague-Dawley (Huntington Farms) rat ileum, as evidenced by the lack of thiol potentiation of the stimulating action of bradykinin on this system.

The only exception encountered with thiol potentiation of bradykinin's stimulant action on the guinea pig ileum was in the case of 2-propanethiol. This could be interpreted on the basis of the chelating potency of the three thiols used. Cysteine and 2-mercaptoethylamine have as functional groups both a thiol and an amine, 2-propanethiol possesses only a thiol. The amine function of cysteine and 2-mercaptoethylamine will enhance their chelating ability and hence account for the observed potentiation. However, 2-propanethiol was found to be as effective as cysteine and 2-mercaptoethylamine in their potentiation of the *in vivo* rat blood pressure.

In addition to a metallo-enzyme mechanism of bradykinin's inactivation, other mechanisms must then exist to explain the inability of cysteine, 2-mercaptoethylamine, and 2-propanethiol to potentiate the stimulatory action of bradykinin on the Sprague-Dawley (Huntington Farms) rat ileum. The carboxypeptidase B-type enzymes may not be the main method of degredation of bradykinin in this rat ileum, and other enzyme systems may be more important in this tissue, such as aminopeptidase, prolidase, or chymotrypsin (1, 17, 18). In support of this suggestion, Feinstein and Ballin (19) investigated the distribution of carboxypeptidase A and B in the Sprague Dawley rat and found those enzymes to be ubiquitously distributed in this animal, including the intestinal tract.

Cirstea suggested in his paper (13) that thiol compounds(thioglycolic acid and cysteine) may cause an increase in the amount of bradykinin receptors "following the rupture of disulfide bridges and the reversible denaturation of the tissue proteins." The results obtained in the present investigation would tend not to support this hypothesis on two counts:

1. The rat ileum failed to respond to a known potentiating agent (cysteine) and other agents that were found to be effective on the rat's *in vivo* blood pressure (2-mercaptoethylamine and 2-propanethiol).

2. 2-Propanethiol could not potentiate the action of bradykinin on the guinea pig ileum.

Table VII—Action of Methionine on Bradykinin's Effect on the Blood Pressure of the Male Rat

Rat No.	Bradykinin Control before Test Agent, mcg.	Pressure[a] Change mm. Hg	Pressure[a] Change % [b]	Test Agent, ml.	Test Agent with Bradykinin Control Pressure Change mm. Hg	Test Agent with Bradykinin Control Pressure Change %
1	0.100	105–90	14.3	0.20	97–83	14.4
		102–90	11.8		90–80	11.1
		105–88	16.2		104–90	13.5
		110–95	13.6		106–90	15.1
2	0.100	88–65	26.1	0.20	85–61	28.2
		86–68	20.9		85–68	20.0
		86–68	20.9		78–62	20.5
		91–72	20.9		100–80	20.0
3	0.100	86–74	14.0	0.20	86–76	11.6
		86–70	18.6		85–68	20.0
		94–78	17.0		85–68	20.0
		95–78	17.9		96–77	19.8
4	0.100	93–85	8.6	0.25	75–70	6.7
		82–75	8.5		88–80	9.1
		89–82	7.9		96–85	11.5
5	0.100	88–76	13.6	0.20	88–74	15.9
		95–76	20.0		95–75	21.1
		101–79	21.8		101–77	23.8
6	0.100	105–78	25.7	0.20	103–73	29.1
		110–85	22.7		115–98	14.8

[a] Change in mean blood pressure. [b] Percent decrease in mean blood pressure.

Methionine was found to be ineffective as a potentiating agent for bradykinin in the rat blood pressure experiments. This amino acid differs from the cysteine used, in not having a free sulfhydryl group present, which indicates that the —SH group is necessary if the compound is to be effective as a potentiator of bradykinin.

SUMMARY AND CONCLUSIONS

1. Cysteine, 2-propanethiol and 2-mercaptoethylamine were found to potentiate the action of bradykinin on the rat blood pressure to varying degrees.

2. Increased doses of bradykinin were chosen to mimic the depression of the thiol-potentiated decrease of the rat blood pressure.

3. The same thiols were found to be ineffective on the *in vitro* rat ileum.

4. The use of cystine was abandoned when it was found that the acid or base needed for its dissolution would cause a depressor response on the rat blood pressure.

5. Experiments on guinea pig ileum were performed to confirm the experimental procedure, and it was found that cysteine and 2-mercaptoethylamine were capable of potentiating the stimulating action of bradykinin. 2-Propanethiol was still found to be ineffective.

6. The results of this investigation tend to support the hypothesis that a carboxypeptidase B-type enzyme may play a major role in terminating the action of bradykinin on rat blood pressure and guinea pig ileum; but in the rat ileum, this does not rule out the involvement of other enzyme systems, such as aminopeptidase, prolidase, or chymotrypsin.

7. The inability of 2-propanethiol to potentiate the effect of bradykinin on the guinea pig ileum may be due to a lack of an amine group, which reduces its ability to chelate the zinc in the active center of the carboxypeptidase.

8. Methionine was found to be ineffective as a potentiator of the effects of bradykinin; this is probably due to the nonavailability of the sulfur group for chelation of zinc.

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