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Research Article

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Inhibition of Vascular Permeability Changes in Rats by Captopril

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ABSTRACT Systemic treatment of rats with captopril (50 mg/kg body wt per os), a specific competitive inhibitor of angiotensin I-converting enzyme, significantly inhibits vascular permeability changes induced by the intradermal injection of the vasoactive mediators histamine, bradykinin, serotonin, and compound 48/80. This effect of captopril is both dose- and time-dependent with ~60% inhibition of edema formation observed 7 h after captopril treatment (100 mg/kg body wt per os). The inhibitory effect of captopril on edema formation is temporally unrelated to the inhibition of serum angiotensin I-converting enzyme activity or serum prostaglandin E₂ levels and is not inhibited by systemic treatment of rats with indomethacin. The data suggest that captopril may have potent antiinflammatory activity through as yet undefined mechanisms.

INTRODUCTION

Angiotensin-converting enzyme (ACE),¹ a dipeptidyl carboxypeptidase, catalyses the conversion of angiotensin I to the octapeptide angiotensin II and has been shown to inactivate bradykinin (1, 2). In recent years several competitive inhibitors of ACE activity have been developed including SQ14225 (3-mercaptopro-2-methylpropanoyl-l-proline). Also known as captopril, SQ14225 has been shown to enhance the hypotensive effects of bradykinin in both rabbits (3) and rats (4). Several authors have suggested that the hypotensive effects of captopril may be the result of increased production of circulating prostaglandins especially of the E series by the kidneys (5).

Recent studies have demonstrated increased serum levels of ACE activity in patients with systemic granulomatous disease including sarcoidosis (6), leprosy (7), and Gaucher's disease (8). In the cases of granu-

lomatous inflammation the increased ACE activity has been associated with epithelioid histiocytes and sarcoid granulomas as well as Gaucher's cells (6, 8). In addition, granuloma isolated from mice infected with *Schistosoma mansoni* show measurable quantities of ACE activity (9). If mice are pretreated with captopril there is a decrease in ACE activity in isolated granulomas as well as a decrease in size of the granulomas. In vitro treatment of macrophages with captopril has also been reported to decrease ACE activity (10). These data suggest that ACE may have a functional role in modulating inflammatory processes.

Several studies have demonstrated that prostaglandins, particularly of the E series (PGE), can modulate both acute and chronic inflammation (11-13). Recent investigations in our laboratory have shown that systemic treatment of rats with PGE₁ or its 15-S-15-methyl-PGE₁ analog can suppress the local vascular permeability changes mediated by vasoactive mediators (14). Since captopril has been demonstrated to increase circulating prostaglandin levels and potentiate the systemic effects of bradykinin, the present investigation was undertaken to examine what effects systemic captopril treatment would have on vascular permeability changes induced by vasoactive mediators in the skin of rats.

METHODS

Animals. Adult male normotensive Sprague-Dawley rats (Charles River Breeding Laboratories, Willmington, MA) weighing ~200-250 g were used in all experiments.

Captopril treatment. Captopril (SQ14225) was a gift of Dr. Zola Horovitz (E. R. Squibb & Sons, Inc., Princeton, NJ). Specific dilutions of the drug were made in phosphate-buffered saline (PBS) so that each animal received 1.0 ml given by mouth. Each rat was fasted overnight receiving only water before captopril administration. The animals were rested 15 min-48 h after captopril treatment before challenge with vasoactive compounds. Multiple skin sites were used on three to five animals for each data point per experiment.

Vasoactive mediators. Histamine dihydrochloride, bradykinin, serotonin creatinine sulfate, and compound 48/80 (Sigma Chemical Co., St. Louis, MO) were diluted in PBS

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¹ Abbreviations used in this paper; ACE, angiotensin-converting enzyme; PBS, phosphate-buffered saline; PGE, prostaglandins of E series; TXB₂, thromboxane B₂.

and edema was induced by the intradermal injection of 0.1 ml with a 27-gauge needle.

Permeability measurements. The intensity of the permeability change and edema formation in each skin site was determined as previously described (14). Briefly, 0.5 μ Ci of 125 I-bovine serum albumin in PBS containing 2.5% Evan's blue dye was administered intravenously before intradermal injection of the mediators. Negative control sites were injected intradermally with 0.1 ml of PBS. At various time points after challenge animals were killed and 1.0 ml of blood and 1.0-cm Diam skin sites were removed and measured for gamma emissions. The permeability index (PI) was calculated as follows: $PI = \frac{^{125}I \text{ emissions per skin lesion}}{^{125}I \text{ emissions per 1.0 ml blood}}$

ACE activity. Serum ACE activity was determined in animals by the production of hippuric acid from hippuryl-L-histidyl-L-leucine as monitored by spectrophotometric assay at 228 nm (15).

Prostaglandin levels. Plasma PGE₂, PGF_{2 α} , and thromboxane B₂ (TxB₂) levels were determined in animals by radioimmunoassay (16-18). The sensitivity of each assay is between 2 to 8 pg/ml plasma.

Statistical analysis. The Student's two-tailed *t* test or analysis of variance were used in comparing treated to non-treated animal groups.

RESULTS

Pretreatment of rats with captopril (100 mg/kg, per os) significantly inhibited the vasopermeability changes induced by bradykinin (Fig. 1). Control animals showed a dose-dependent increase of permeability index after intradermal injection of bradykinin reaching a peak value of 0.20 ± 0.03 (approximately fourfold increase over control values) at a dose of 20 μ g. However, in captopril-treated animals there was only a slight

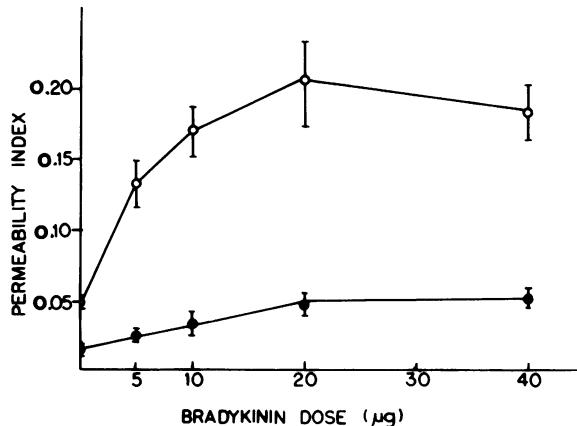


FIGURE 1 Effect of captopril treatment on bradykinin-induced vasopermeability changes. Captopril (100 mg/kg, per os) was administered 20 min before intradermal challenge with bradykinin. Animals were killed 15 min after bradykinin injection and the permeability index determined (Methods). Each data point represent mean values from three to five rats \pm SEM. ○, control, nontreated animals; ●, captopril-treated animals.

increase in the permeability index with increasing doses of bradykinin, remaining below a value of 0.05 at all bradykinin doses up to 40 μ g. In this experiment captopril was administered 20 min before challenge with bradykinin, and the animals were killed at 15 min after bradykinin injection.

In additional experiments the effects of systemic captopril treatment on vasopermeability changes induced by other vasoactive mediators including histamine, compound 48/80, and serotonin were examined. Pretreatment of animals with captopril (100 mg/kg) 20 min before mediator challenge produced $>70\%$ ($P < 0.025$) inhibition of edema formation by each of the mediators tested (Table I). Captopril treatment produced significant inhibition of vascular permeability changes induced by each of the four mediators tested up to 7 h after captopril treatment. However, at 18 h after captopril treatment there was no significant inhibition of edema formation.

The dose dependence of captopril-induced inhibition of vascular permeability changes is shown in Table II. When animals were treated with captopril 20 min before challenge with vasoactive mediator at a concentration of 50 mg/kg or 100 mg/kg there was significant inhibition ($>70\%$, $P < 0.025$) of edema formation. However, when the concentration of captopril was decreased to 10 mg/kg there was only $\sim 40\%$ inhibition of edema formation with bradykinin and compound 48/80, while there was no significant inhibition of histamine-induced edema formation. When captopril was administered at a dose of 1 mg/kg (data not shown) there was no inhibition of edema formation by any of the three mediators tested. The difference in inhibition of edema formation between histamine, bradykinin, and compound 48/80 at a dose of 10 mg/kg captopril may reflect the difference in rat sensitivity to these mediators (19).

In an effort to assess the *in vivo* ACE-inhibiting activity of captopril, serum ACE activity was determined after treatment with three concentrations of captopril. 100 mg/kg of captopril produced a reduction of serum ACE activity of $\sim 80\%$ at 0.5 h after oral administration (Fig. 2). This activity remained significantly depressed up to 4 h (29.6% decrease, $P < 0.05$) after captopril treatment. When the captopril dose was decreased to 50 mg/kg serum ACE activity was also significantly decreased up to 4 h (32.2% decrease, $P < 0.05$) after treatment. The dose-dependent effect of captopril treatment on serum ACE activity was manifest by the 49.1% ($P < 0.01$) reduction of ACE activity at 0.5 h and only 7.9% reduction at 4 h after captopril treatment with 10 mg/kg. At 6 h after captopril treatment there was no significant decrease in serum ACE activity at any of the three doses. This data demonstrates that oral treatment of rats with cap-

TABLE I
Time Dependence of Captopril-induced Inhibition of Vascular Permeability

Dose	Percent inhibition of vascular permeability					
	20 min	P	7 h	P	18 h	P
μg						
Histamine	40	70.2 \pm 5.5	<0.025	54.8 \pm 13.4	<0.05	0.0 \pm 20.3
Bradykinin	20	73.2 \pm 1.9	<0.025	64.3 \pm 11.5	<0.02	24.7 \pm 20.1
Compound 48/80	40	69.8 \pm 6.6	<0.01	71.7 \pm 5.7	<0.01	38.6 \pm 20.8
Serotonin	20	80.5 \pm 3.2	<0.01	60.8 \pm 11.6	<0.01	26.7 \pm 18.0

Data represents mean \pm SEM.

Captopril (100 mg/kg, per os) was administered at 20 min, 7 h, and 18 h before intradermal challenge with vasoactive mediators. At 15 min after injection of vasoactive mediators the animals were killed and a permeability index (Methods) was determined for each mediator in captopril-treated animals. Mean values were determined from three to five animals and compared to permeability changes in control animals. The percent inhibition of vascular permeability was calculated and P values determined by the Student's two-tailed t test.

topril produces a transient inhibition of serum ACE activity beginning as early as 0.5 h after treatment and returning to control levels by 6 h after treatment. This effect is in contrast with the prolonged inhibition of vascular permeability changes that remained significantly suppressed at 7 h after captopril administration.

Since captopril has been shown to increase circulating prostaglandin levels, especially PGE₂ (5), the concentration of three arachidonic acid metabolites, PGE₂, PGF_{2 α} , and TxB₂ were determined in plasma at 30 min after captopril treatment. At a dose of 100 mg/kg, captopril produced a significant increase in circulating PGE₂ (43.2%, P < 0.025) and PGF_{2 α} (47.5%, P < 0.025) levels above control values and only a small increase in circulating TxB₂ levels (23.6%, P < 0.25) (Table III). When the dose of captopril was reduced to 50 mg/kg there was no significant increase in circulating PGE₂, PGF_{2 α} , and TxB₂, levels at 30 min after treatment. This contrasts with the effects of captopril on the inhibition of vascular permeability, since a 50-

mg/kg dose of captopril produced >70% inhibition of vasoactive mediator-induced edema. In addition, when animals were pretreated with indomethacin (20 mg/kg, i.p., in two doses 12 h and 1 h before captopril treatment, data not shown) there was no alteration in the captopril-induced inhibition of vascular permeability by vasoactive mediators. In fact, indomethacin at this dose appeared to mildly potentiate the inhibitory effects of captopril.

DISCUSSION

ACE can modulate blood pressure through the regulation of the conversion of angiotensin I to angiotensin II (1). In addition ACE has been shown to enzymatically inactivate the potent vasoactive mediator bradykinin (2). Recently, a series of competitive inhibitors of ACE activity have been developed as antihypertensive agents (20). Captopril, along with other competitive inhibitors of ACE activity have been

TABLE II
Dose Dependence of Inhibition of Vascular Permeability by Systemic Captopril Treatment in the Rat

Dose	Captopril dose, percent inhibition of vascular permeability					
	10 mg/kg	P	50 mg/kg	P	100 mg/kg	P
μg						
Histamine	40	5.1 \pm 12.1	NS	80.6 \pm 0.3	<0.025	70.2 \pm 5.5
Bradykinin	20	44.3 \pm 11.9	<0.05	73.9 \pm 2.1	<0.01	73.2 \pm 1.9
Compound 48/80	40	41.5 \pm 10.0	<0.01	77.6 \pm 1.8	<0.01	69.5 \pm 6.6

Data represents mean \pm SEM.

Captopril (per os) was administered at varying doses 20 min before challenge with vasoactive mediators. Animals were killed 15 min after vasoactive mediator injection and the percent inhibition of vascular permeability changes determined as described in Table I.

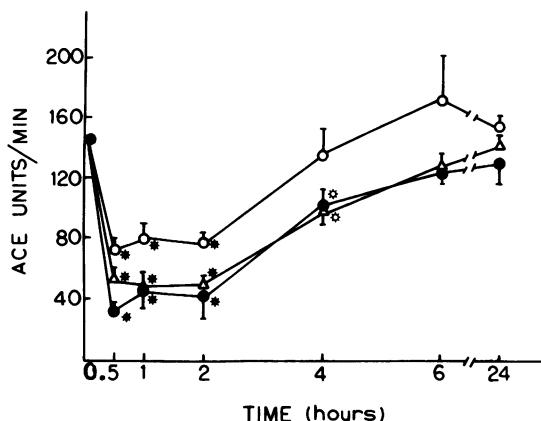


FIGURE 2 Effect of captopril treatment on serum ACE activity. Serum ACE activity was determined at various times after captopril treatment. Each data point represents mean values from three to five rats \pm SEM. ●, captopril-treated animals (100 mg/kg); Δ, captopril-treated animal (50 mg/kg); ○, captopril-treated animals (10 mg/kg).

* $P < 0.01$. * $P < 0.05$.

shown to lower blood pressure in both rats (4) and humans (5).

Increased levels of ACE activity in serum and isolated cell preparations have been associated with various granulomatous diseases including sarcoidosis (6), leprosy (7), Gaucher's disease (8), and systemic murine schistosomiasis (9). In vitro studies have shown that treatment of macrophages with captopril will decrease ACE activity (10). Recent in vivo experiments have shown that systemic treatment of mice with captopril will inhibit both BCG-induced lung granulomas (21) and reduce granuloma formation in an experimental model of murine schistosomiasis (9). Because one of

the initial responses of a host tissue to injury is an increase of vascular permeability leading to exudation of plasma components and edema formation, and because ACE is known to inactivate bradykinin, we examined the effects of systemic captopril treatment of rats on vasoactive mediator-induced vasopermeability. The data demonstrates that per os treatment of rats with captopril will inhibit the vascular permeability changes induced by the vasoactive mediators histamine, serotonin, and bradykinin. In addition, edema formation induced by compound 48/80, which causes mast cell degranulation, is also inhibited. The inhibition of edema by captopril is both dose- and time-dependent with a maximum effect of captopril observed at \sim 20–30 min after treatment at a dose of 50 mg/kg. Although relatively high doses (compared with effective antihypertensive doses) of captopril (50 mg/kg, per os) were necessary to elicit a maximal inhibitory effect on vascular permeability changes, the doses of captopril used in this study are comparable or less than those doses used in other studies that showed inhibition of inflammatory responses by captopril (9, 21). Additional studies examining a variety of the newly developed inhibitors of ACE activity may identify individual compounds that show greater specificity for antiinflammatory effects vs. antihypertensive activity.

The mechanism by which captopril inhibits vascular permeability changes induced by vasoactive mediators is not clear. Because ACE breaks down bradykinin, it would be expected that treatment of rats with captopril and the resulting inhibition of ACE activity would result in a potentiation of bradykinin-induced edema formation. This finding is in contrast with the inhibition observed in these experiments. In addition, the inhibition of vascular permeability changes by captopril persisted for at least 7 h after treatment, while the serum ACE activity was not significantly depressed at 6 h after captopril treatment. However, these studies do not exclude the possibility that tissue ACE activity was decreased for prolonged periods compared with serum ACE activity.

The possibility that the inhibition of edema formation was a direct result of the hypotensive effects of captopril was considered. However, experiments in normotensive rats have shown that per os treatment with captopril in doses up to 100 mg/kg per d causes only a transient 10% depression in mean arterial blood pressure, where saline control animals experienced a 6% decrease (22). Therefore it would be unlikely that this 4% (\sim 5 mm Hg) transient decrease in mean arterial blood pressure would account for the profound inhibition of edema formation. In addition, the rats showed normal behavior activity during captopril treatment and the inhibition of edema formation persisted for at least 7 h after treatment.

Recent studies in our laboratory have shown that

TABLE III
Effect of Captopril Treatment on Plasma PGE₂, PGF_{2α}, and Tx_{B2} Levels in the Rat

	Captopril treatment						
	50 mg/kg		100 mg/kg				
	Control	Percent increase above control					
<i>pg/ml*</i>							
PGE ₂	782 \pm 33	10.3	NS	43.2	$P < 0.025$		
PGF _{2α}	320 \pm 13	14.1	$P < 0.2$	47.5	$P < 0.025$		
TxB ₂	310 \pm 56	-15.1	NS	23.6	$P < 0.25$		

* Data represent mean \pm SEM.

Captopril (per os) was administered at varying doses to three to five rats and the animals killed 30 min later. Plasma samples were assayed for PGE₂, PGF_{2α}, and Tx_{B2} levels by radioimmunoassay. These levels were compared with control animals and the percent increase above control levels determined. P values were determined by Student's two-tailed t test.

systemic treatment of rats with prostaglandins of the E series can inhibit various inflammatory reactions (11-13) as well as edema formation caused by vasoactive mediators (14). Since captopril is known to increase circulating PGE₂ levels (5) and indomethacin treatment can inhibit its hypotensive effects (3), we tested the possibility that captopril-induced inhibition of vascular permeability is a result of increased plasma PGE₂ levels. At high doses of captopril (100 mg/kg) there was significant elevation of rat PGE₂ levels. At 50 mg/kg captopril continued to block edema formation, however, there was only a small nonsignificant increase (10.3%) in plasma PGE₂ levels. In addition, pretreatment of rats with indomethacin (20 mg/kg) failed to inhibit the effects of captopril on vascular permeability. Therefore, we could not establish a definitive role for prostaglandins in this response. Although some studies report increased plasma prostaglandin levels in captopril-treated animals, our observations are consistent with others who have failed to show a relationship between serum PGE₂ levels and captopril's antihypertensive effects (23).

In summary, the data presented here demonstrate that systemic treatment of rats with captopril will inhibit vasoactive mediator-induced edema formation. The evidence suggests that captopril may have potent antiinflammatory activity and potential as an antiinflammatory drug. However, the precise mechanism by which captopril inhibits edema formation is not known.

ACKNOWLEDGMENT

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