Hypotensive Effect of Captopril
Role of Bradykinin and Prostaglandinlike Substances
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Captopril (0.15–10 mg/kg) administration in the anesthetized dog causes immediate hypotension concomitant with an increase in tonus of the assay tissue (cat terminal ileum) superfused with circulating blood (Vane's cascade method). The increase in cat terminal ileum tonus was antagonized by a bradykinin receptor antagonist, L-349b. Treatment of the animals with indomethacin blocked or reversed the hypotensive effect of captopril without affecting the increase in tonus of the cat terminal ileum. Captopril potentiated the hypotension induced by bradykinin injected intra-arterially, and indomethacin reduced the hypotensive effect of intra-arterially injected bradykinin. Addition of captopril or enalapril to the superfusing blood maintained at 37° C in an extracorporeal circuit caused a long-lasting increase in the tonus of the cat terminal ileum. The present results support the hypothesis that immediate hypotension induced by captopril involves a prostaglandin-dependent component possibly resulting from increased bradykinin levels generated in the vicinity of captopril action. (Hypertension 1990;15(suppl I):I-55–I-58)

There is considerable evidence in support of a mechanism in addition to that through which inhibition of angiotensin II generation in the blood accounts for the antihypertensive action of the various converting enzyme inhibitors. In brief, essential hypertension and several experimental models of hypertension not dependent on the renin-angiotensin system are effectively controlled by captopril1-3; inhibition of plasma angiotensin converting enzyme does not always correlate with its hypotensive effect.4-6 Kinin antibodies are known to block the increases in uteroplacental blood flow and plasma prostaglandin E2 induced by converting enzyme inhibitors in pregnant, nephrectomized rabbits.7,8 Because the same enzyme is responsible for angiotensin conversion and bradykinin (BK) inactivation, increased BK levels might partially explain the hypotensive activity of the converting enzyme inhibitors. It is noteworthy that the reduction in blood pressure of renovascular hypertensive rats by enalapril is significantly blocked by the infusion of a BK antagonist, whereas no effect is observable when the pressure decrease is induced by saralasin or nitroprusside.9 There have been several demonstrations that BK releases cyclooxygenase metabolites from various tissues.10 In the normal dog, the hypotensive effects of captopril and BK are significantly reduced by indomethacin, thus suggesting that the acute hypotensive effect of captopril in the dog might result from the local release of prostacyclin, stimulated by increased plasma BK levels.11 In the present study, we reinvestigate the blockade of the hypotensive effect of captopril by indomethacin and the relation with plasma BK levels, measured by the blood-bathed bioassay technique of Vane.12 The presence of BK in the circulating blood was confirmed by blocking the response of the assay tissues with a specific BK receptor antagonist, L-349b.13

Methods
Mongrel dogs of either sex, weighing 12–15 kg, were anesthetized with pentobarbital sodium (30 mg/kg i.v.). After intubation, the animal was allowed to breathe spontaneously. Intravenous injections were made through a cannula inserted into the femoral vein. Blood pressure and heart rate were measured by a pressure transducer (P23Db, Gould Statham, Oxnard, California) attached to a catheter inserted into the left femoral artery and recorded on a Beckman polygraph recorder (R611, Beckman Instrs., Schiller Park, Illinois). The dogs were given heparin (1,000 IU/kg i.v.). Arterial blood, continuously withdrawn using a roller pump at a rate of 10 ml/min from a catheter inserted into the right femoral artery, was used to superfuse a series of bioassay tissues for the detection of BK. A catheter inserted into the right femoral vein was used to return the blood from the cascade to the dog. Intra-arterial injections of BK were made through a cannula inserted into the ascending aorta. Three longitudinal strips of cat terminal ileum (CTI)14 were used for the BK bioassay. Before

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blood superfusion, the assay tissues were superfused with Krebs solution that was gassed with 95% O2 and 5% CO2 and contained a mixture of antagonists (hyoscine, mepyramine, methysergide, propranolol, phenoxybenzamine,13 and indomethacin) for 2 hours to render them more selective and sensitive to BK. Alteration in the length of the assay tissues was detected by auxotonic transducers (Heart/Smooth Muscle Transducer 386, Harvard Apparatus, South Natick, Massachusetts), the output of which was displayed on a Beckman polygraph (R611, Beckman Instrs., Inc.). The presence of BK was determined by comparison of the biological activity of the substance released with that produced by authentic BK administered directly into the blood superfusing the tissues.

In four experiments, the extracorporeal circuit (volume, 90 ml) superfusing the bioassay tissues was extended with silicone tubing, maintained at 37°C in a bath, and disconnected from the animal. Captopril (two experiments) or enalapril (two experiments) was added to this circuit at a final concentration of 1.5 μg/ml.

The following drugs were used: bradykinin triacetate (Sigma-Aldrich, St. Louis, Missouri), histamine (Sigma-Aldrich), enalapril (Merck Sharp & Dohme, Division of Merck & Co., Inc., West Point, Pennsylvania), captopril (E.R. Squibb & Son, Princeton, New Jersey), indomethacin (Merck Sharp & Dohme), hyoscine hydrobromide (Burroughs Wellcome, Beckenham, Kent, England), mepyramine maleate (Anthisan, May & Baker, Dagenham, England), methysergide bimaleate (Sandoz Pharmaceuticals, East Hanover, New Jersey), phenoxybenzamine hydrochloride (Smith, Kline & French Laboratories, Philadelphia, Pennsylvania), propranolol hydrochloride (Sigma-Aldrich), L-349b B4307 (102-159) [DArg*]Hyp3-Thi5-8[DPhe7]BK (John M. Stewart, Department of Biochemistry, University of Colorado, Medical School, Denver, Colorado).

**Results**

High (10 mg/kg, n=6) and low doses (150 μg/kg, n=11) of captopril injected intravenously into normotensive dogs caused hypotension concomitant with an increase in the tonus of the CTI superfused with circulating blood. The mean arterial blood pressure decreases were 33.0 ± 24.0 mm Hg and 19.5 ± 1.4 mm Hg, respectively, for the high and low doses of captopril. Although there is a clear difference in hypotensive effect of the two concentrations used, there was no comparable variation in the tonus of the CTI. The increase in tonus of the CTI after captopril injection was equivalent to that induced by 3-5 ng/ml BK directly over the CTI before the administration of captopril to the animals or during contractions of CTI induced by the infusion of histamine (4-6 ng/ml, n=3). Treatment of the animals with indomethacin (2 mg/kg, n=17) reversed the hypotensive effect of captopril without affecting the increase in tonus of the CTI, as illustrated in Figure 2. Hypotension was not observed when the animals were pretreated with indomethacin (n=4) before captopril administration (10 mg/kg), although there was an increase in the CTI tonus equivalent to that observed in the nontreated animals. In two animals, the action of indomethacin was tested regarding the potentiation by captopril of the hypotensive effect of BK (1 μg) injected into the ascending aorta. Captopril potentiated both the intensity and the duration of the BK hypotensive effect, whereas indomethacin markedly reduced this effect, that is, the short-lived effect of BK (15-25 seconds) was increased to 15-20 minutes' duration after captopril (150 mg/kg), whereas the treatment of the animals with indomethacin (2 mg/kg) reduced this effect to 3-4 minutes.

There was an immediate increase in CTI tonus after captopril, which persisted until the end of the observation period (40-60 minutes); this increase was unaffected by indomethacin and was equivalent to that induced by 3-5 ng/ml BK.

**Discussion**

The present series of experiments confirm that treatment with captopril of normotensive animals, specifically dogs anesthetized with pentobarbital, as used here, causes an immediate long-lasting hypotension. A hypothetical blockade of the activation of the renin-angiotensin system caused by surgery, anesthesia, or both has been evoked to explain the systemic blood pressure effect of this converting enzyme inhibitor. An additional component, that is, the release of prostacyclin by the kidneys due to increased BK levels in the renal circulation, should also be considered. This hypothesis is supported by the facts that 1) BK infused in the kidney releases prostacyclin, 2) captopril potentiates BK-induced hypotension, and 3) the subsequent treatment of the dog with indomethacin abolishes renal release of prostacyclin and reduces the BK-induced systemic hypotensive effect.

Mullane and Moncada also have described an inconstant increase in the tonus of blood-bathed assay tissues (rat stomach strip and CTI) after captopril administration, interpreted as an increase in plasma BK level. In all our experiments, administration of captopril caused an increase in CTI tonus. This increase in tonus might have been due to the presence of BK in the blood because 1) captopril did not cause contraction of the CTI superfused with Krebs solution, 2) the assay tissues were pretreated with a
multitude of antagonists (antihistaminic, antiserotonergic, parasympatholitic, sympatholitic, and cyclooxygenase inhibitor), and 3) infusion of a specific, competitive BK antagonist, L-349b, produced a pronounced reduction in the increase in CTI tonus. In support of the hypothesis that the release of a cyclooxygenase metabolite secondary to an increase in plasma BK concentration might be partially responsible for the acute captopril-induced hypotension, our data show that indomethacin 1) reduced the intensity of the captopril-induced potentiation of hypotension resulting from intra-arterially injected BK and 2) abolished captopril-induced hypotension without affecting the appearance of BK in the blood.
The principal question at this point is whether this acute hypotensive effect of converting enzyme inhibitors, apparently mediated by the release of cyclooxygenase metabolites, is relevant in terms of the antihypertensive effect of these drugs. There are contradictory reports describing this phenomenon in the dog and other species investigated. There is an apparent discrepancy in the fact that low and high doses of captopril produce different effects on systemic blood pressure, whereas the amount of kinin detected remains essentially unchanged. This is explainable considering that the detection of BK by the assay tissue reflects the presence of plasma kininase, which is fully inhibited by small doses of captopril. The hypotensive effect, however, depends not only on circulating kininase but also on the inhibition of the enzyme in the tissues, which is only achieved at higher concentrations of the inhibitor.

Finally, the fact that BK was consistently detected in the circulating blood after captopril treatment raises the question of whether continuous, low-level formation of BK occurs in the circulating blood, the presence of which is only revealed by inhibition of kininase II (angiotensin converting enzyme). To test this hypothesis, we used an extracorporeal circuit to superfuse the tissues with blood; treatment with captopril or enalapril produced a persistent increase in the tonus of the CTI. An artificial activation of kinin formation by the incubating circuit is improbable because the activation of the plasma kinin system by a foreign surface is generally short-lived, possibly due to the adsorption of plasma proteins. The formation of BK-like substances in this extracorporeal circuit also was not due to an atypical activation of the system by captopril because the same effect was observed with another angiotensin converting enzyme inhibitor, enalapril. Because tonus in the assay tissue reached a plateau after the addition of either captopril or enalapril to the extracorporeal circuit, it is assumed that continuous inactivation of the BK formed is brought about by enzymes other than kininase II. This might be one of the reasons why the normal BK level in the blood does not increase after the treatment of patients or animals with angiotensin converting enzyme inhibitors, although it has been shown that converting enzyme inhibitors in the rat cause an increase in the level of kinin in the blood.

The present results are consistent with the following hypothesis: Continuous formation of BK occurs in the circulating blood at a level maintained very low by the various kininases present in the plasma, tissues, and blood cells. Captopril, by inhibiting kininase II, blocks angiotensin I conversion to the active hypertensive peptide, angiotensin II. Captopril also increases the local BK levels that affect vascular resistance either directly or indirectly by releasing cyclooxygenase vasodilator metabolites from the kidneys, the vessels themselves, or both. Should this hypothesis be correct, the local increase in BK levels might represent an additional factor in the lowering of peripheral resistance or in increasing renal salt excretion.

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