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The effects of *S*-nitrosocaptopril on renal filtration and blood pressure in rats

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Abstract

The present investigation was performed to evaluate the effects of *S*-nitrosocaptopril, a novel vasodilator possessing the capacities of both an angiotensin converting enzyme inhibitor and an NO donor, on blood pressure and renal function in rats. *S*-nitrosocaptopril produced acute reductions in mean arterial pressure after both oral dosing (5, 10, 50 mg/kg) to chronically-catheterized awake rats and intravenous administrations (0.125, 1.25, 12.5 mg/kg) to anesthetized rats. The hypotensive magnitude and duration of *S*-nitrosocaptopril were dose-dependent. Acute pressure-associated reductions in the glomerular filtration rate and urine flow were observed only at high concentration of *S*-nitrosocaptopril (12.5 mg/kg, i.v.) in both awake and anesthetized rats. These decreases were transient, followed by an overshoot of glomerular filtration rate and urine flow above basal values. In contrast, captopril (i.v.) did not produce any significant acute effects on mean blood pressure and glomerular filtration rate in either awake or anesthetized rats. In rats with acute hypertension induced by N^G-monomethyl-L-arginine (L-NMMA, 30 mg/kg, i.v.), *S*-nitrosocaptopril (0.125 mg/kg, i.v.) significantly abolished the hypertensive effects. In contrast, the hypertension was not affected by captopril. In two-kidney one-clipped Goldblatt hypertensive rats, oral administration of *S*-nitrosocaptopril (25 mg/kg, b.i.d.) for 10 days significantly reduced systolic blood pressure and preserved glomerular filtration rate. The oral antihypertensive effect of *S*-nitrosocaptopril was more potent than captopril ($P < 0.05$). In conclusion, these findings indicate that: (1) *S*-nitrosocaptopril provides both acute and chronic anti-hypertensive effects orally and intravenously, whereas captopril has only moderate chronic oral effects; and (2) *S*-nitrosocaptopril preferentially decreases blood pressure without markedly affecting glomerular filtration rate.

Keywords: *S*-Nitrosocaptopril; Captopril; Nitric oxide (NO); Glomerular filtration rate; Mean arterial pressure; Glodblatt hypertensive rat

1. Introduction

S-nitrosothiols have been proposed as possible storage forms of endothelium-derived nitric oxide (NO) (Ignarro et al., 1981; Myers et al., 1990; Jia et al., 1996), a naturally occurring substance that participates in the control of vascular tone and in renal hemodynamics, among other functions such as antioxidant and antibacterial properties (Incze et al., 1984). Evidence from animal studies using NO synthase inhibitors to impair the L-arginine/NO pathway suggest that NO is an important regulator of cardiovascular and renal hemodynamics, sodium excretion, and renin release (Blantz, 1994). Administration of NO inhibitors in rats has shown a decrease in renal blood flow, glomerular filtration rate and sodium excretion. Despite the compelling evidence that the L-arginine/NO pathway is ubiquitous in biological systems and the several steps in the pathway are amenable to manipulation, the importance of NO in regulation of renal function, however, has not so far been directly demonstrated and delineated. Very few experimental data have been provided to establish the direct pharmacological influence of NO and *S*-nitrosothiols on cardiovascular and renal hemodynamics. The determination of NO involvement in renal hemodynamics should be mainly based on strict pharmacological criteria, among which is the ability to mimic an endogenous response by administration of authentic NO or a *S*-nitrosothiol, the latter behaves much closely to the authentic NO (Myers et al., 1990; Jia and Furchgott, 1993). Such an approach can also provide important insights into mechanisms of NO effects.

There is increasing interest in compounds which generate NO in a controlled manner. In view of the short

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half-life of authentic NO in vitro (Jia and Furchgott, 1993), the existence of more stable transport forms of NO has been postulated. Prime candidates for such carrier molecules are low molecular mass thiols and protein sulfhydryl groups. The formation of *S*-nitroso proteins induced by NO under physiological conditions has been established for hemoglobin (Jia et al., 1996; Stamler et al., 1997), serum albumin, and tissue-type plasminogen activator (Stamler et al., 1992). *S*-nitrosothiols might serve as good NO carriers since they concomitantly bear their parent thiol bioactivities. They are believed to decompose nonenzymatically to give the corresponding disulfide and NO (Mathews and Kerr, 1993; Singh et al., 1996). Among these *S*-nitrosothiols, *S*-nitrosocaptopril has come to prominence not only because it could in principle be used as part of NO-producing drugs, but also the captopril disulfide, which formed after thermally-induced homolytic cleavage of the S–N bond under physiological conditions, has already exhibited an antihypertensive activity and inhibition of angiotensin converting enzyme in vivo in spontaneously hypertensive rats (Kripalani et al., 1980), presumably because of metabolic conversion of the disulfide to reduced captopril, and to mixed disulfide with glutathione or L-cysteine. Indeed, *S*-nitrosocaptopril was found to possess the ability to inhibit in vitro angiotensin converting enzyme and platelet aggregation, and relax in vitro blood vessels (Loscalzo et al., 1989; Aman et al., 1994). Hence, *S*-nitrosocaptopril has potential clinical implication in the treatment of many forms of cardiovascular diseases as a combined NO donor and an angiotensin converting enzyme inhibitor.

Using isolated blood vessels (Loscalzo et al., 1989) and anesthetized dogs (Shaffer et al., 1991; Nakae et al., 1995), *S*-nitrosocaptopril has been demonstrated as a potent direct vasodilator. As of this writing, however, only the acute effects of i.v. administrated *S*-nitrosocaptopril on the blood pressure in anesthetized dogs have been reported (Shaffer et al., 1991; Nakae et al., 1995). No information is available concerning the oral activity of *S*-nitrosocaptopril on blood pressure in awake animals and its impact on renal filtration when given as a NO donor. The purpose of the present study was therefore to examine the effects of oral doses of *S*-nitrosocaptopril on rat blood pressure, to further investigate the direct effects of *S*-nitrosocaptopril on renal filtration. In addition, the therapeutic effects of oral doses of *S*-nitrosocaptopril on two-kidney, one-clipped Goldblatt hypertensive rats were examined in comparison with the corresponding parent compound captopril.

2. Methods and materials

2.1. Awake rat preparation

All animal experiments in the studies were performed according to the National Institutes of Health guidelines and approved by the University's Institutional Animal Care and Use Committee. Male Wistar rats (body weight 302 ± 18 g) were given Brevital (E. Lilly, Indianapolis, IN, USA) for anesthesia (70 mg/kg, i.p.), and surgery was conducted under full sterile technique. Tygon catheters were implanted in the left femoral artery and vein, and tunneled subcutaneously, exteriorized between the scapulae, and placed in neck collars. In addition, the urinary bladder was implanted with PE 200 to allow urine collection from the kidney into plastic tubes. Catheters were primed and plugged, and after recovery from general anesthesia, rats were returned to individual cages with free access to food and drinking water. The chronically catheterized awake rats were prepared either for blood pressure measurement or for renal function experiments. On the day of renal function experiments, rats were placed in a restraining cage, the bladder pin was removed for collection of urine, the femoral vein catheter was used for the infusion of perfusion solution, and the arterial catheter was connected to a pressure transducer and blood pressure recorder for measurement of mean arterial pressure and blood sampling. On the day of blood pressure measurement, awake rats were fasted for 4 h before experiments, and then were placed in a restraining cage, only the arterial catheter was connected to a pressure transducer for measurement of mean arterial pressure. After baseline of mean arterial pressure was obtained, *S*-nitrosocaptopril or captopril solutions were given by gavage, and a continual pressure recording was performed.

2.2. Anesthetized rat preparation

Male Wistar rats were anesthetized and surgically prepared as described in standard procedures (Blantz et al., 1985). Briefly, rats underwent tracheostomy (PE 200) on a temperature controlled table, and cannulation of the left jugular vein, left femoral artery (both PE 50), and bladder (PE 90). The femoral artery catheter was used for periodic blood sampling and monitoring of the MAP with a transducer (Model P23DB; Statham Instruments, Gould Division, Hato Rey, Puerto Rico) and recorded on a Statham chart recorder. The jugular vein was used for the infusion of perfusion solution. Subsequent to completion of the surgical preparation and before beginning the clearance studies, the rats were allowed to stabilize for 60 min.

2.3. Clearance studies

A continuous intravenous infusion was supplied of perfusion solution (isotonic $NaCl–NaHCl_3$ containing [^{3}H]inulin (5 μCi/ml)) at a rate of 10 μl/min per 100 g rat body weight. This is a nonexpanding infusion rate which approximately equals urine output in our preparation. After 1 h equilibration period, control observations were begun, and three 20-min urine collections were obtained in preweighed containers under oil, and the volume of urine

samples was measured gravimetrically. Blood samples were obtained at the beginning and end of each urine collection. The blood was centrifuged, hematocrit documented, and plasma was utilized to determine [^{3}H]inulin concentration. After completion of control measurements, rats received an intravenous bolus of saline (0.1 ml/kg) containing *S*-nitrosocaptopril at doses of 0.125, 1.25, or 12.5 mg/kg, and then three clearance periods of 20-min and mean arterial pressure were observed at each different dose of a drug. All clearance calculations are based on standard formulas using the mean value of the two plasma levels before and after each clearance period (Blantz et al., 1985). A continuous infusion of [^{3}H]inulin was made throughout the entire experiments except for the short duration of bolus administration (1 min). [^{3}H]inulin activity was measured in 10-μl samples of urine and plasma (in 0.2 ml H_2O + 3 ml scintillation fluid) using a liquid scintillation counter (Tri-Carb 4530, Packard). Glomerular filtration rate was calculated as the urine-to-plasma inulin concentration ratio x urine flow and was factored per gram kidney weight.

2.4. Preparation of two-kidney one-clipped hypertensive rats

The two-kidney one-clipped hypertensive model was induced in male Wistar rats (180 ± 8 g body weight) by placing a 0.2 mm slit width silver clip on the left renal artery under Brevital anesthesia (De Nicola et al., 1990). The contralateral kidney was left intact. Systolic blood pressure was measured weekly in awake clipped animals using an electrosphygmomanometer with tail cuff, pulse transducer and heated restraining cages (Narco Biosystems, Houston, TX, USA). Some 4 to 5 weeks after clipping, time-related hypertension had developed over the observation period, and hypertensive rats were randomly divided into the three different groups ($n = 6$ per group): hypertensive untreated rats, and hypertensive rats treated with either *S*-nitrosocaptopril or captopril (both 25 mg/kg, p.o., b.i.d.). The solution of the compounds were freshly prepared. After 10 days of continuous administration of *S*-nitrosocaptopril or captopril by gavage, these rats were studied by clearance determination.

2.5. Materials

Most of chemicals were purchased from Sigma Chemical (St. Louis, MO, USA). N^G-monomethyl-L-arginine (L-NMMA) was purchased from Calbiochem-Novabiochem, (La Jolla, CA, USA). The preparation of stable pink–red *S*-nitrosocaptopril was accomplished via a nitrosation reaction of captopril (Loscalzo et al., 1989) via the adjustment of appropriate concentrations of reaction medium. This modified procedure provides a more convenient method to produce the target crystals in good yield (Lin et al., 1998).

2.6. Statistical analyses

Unless otherwise noted, all results are presented as mean ± S.E. In some figures, only one S.E. is shown

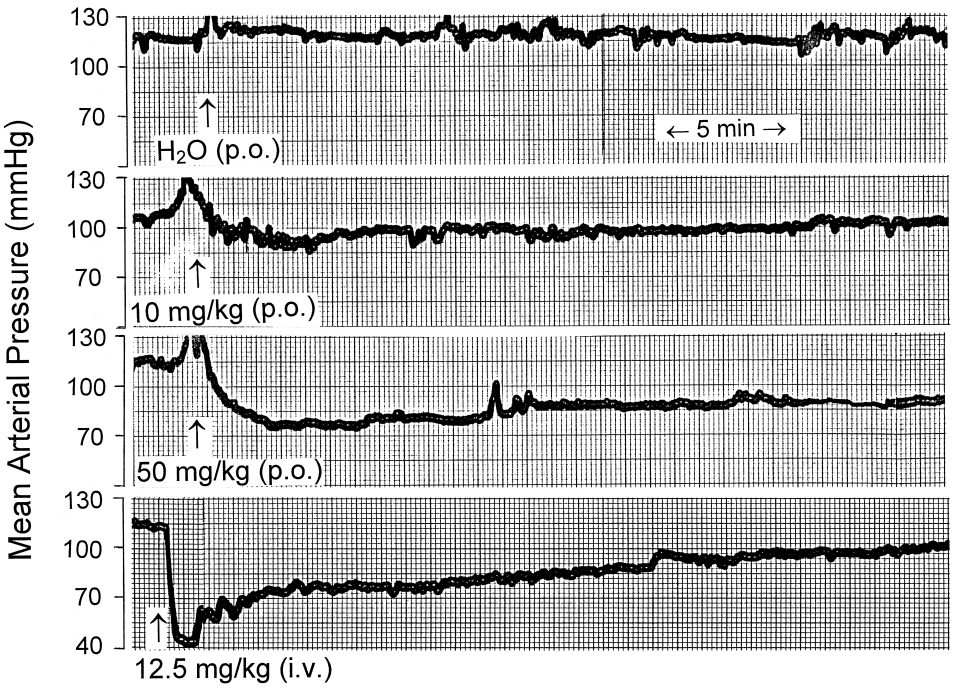

Fig. 1. Dose–response tracings after oral and intravenous administration of *S*-nitrosocaptopril to chronically-catheterized awake rats in restrained cages. Arrows indicate beginning of *S*-nitrosocaptopril administration by gavage (p.o.) or a bolus injection (i.v.) to the rats as recording is performed. Tracings represent five experiments.

either above or below the mean to improve clarity. Differences from control values were analyzed by Student's *t*-test for paired data. Responses to *S*-nitrosocaptopril and captopril in the therapeutical experiment on two-kidney, one-clipped hypertensive rats were compared by a one-way analysis of variance. A P value less than 0.05 was accepted as significance.

3. Results

3.1. Effects of oral doses of S-nitrosocaptopril on blood pressure in awake rats

Oral administration of *S*-nitrosocaptopril by gavage to chronically-catheterized awake rats produced a reduction in mean blood pressure from 112 ± 7 to 99 ± 9 mmHg (5 mg/kg), from 105 ± 5 to 93 ± 7 mmHg (10 mg/kg), and from 110 ± 6 to 74 ± 7 mmHg (50 mg/kg). The hypotensive effects lasted for 29 ± 10 min, 50 ± 8 min, and 99 ± 12 min, respectively. As shown in Fig. 1, *S*-nitrosocaptopril 50 mg/kg produced a marked, prompt and sustained hypotension. In contrast, both vehicle (H_2O) and captopril (50 mg/kg, p.o.) did not exhibit significant effects on blood pressure in awake rats.

3.2. Effects of intravenous doses of S-nitrosocaptopril on blood pressure and glomerular filtration rate in awake rats

Intravenous administration of *S*-nitrosocaptopril immediately resulted in hypotensive responses in chronically catheterized awake rats. Mean arterial pressure fell to the nadir within 5 min, reflecting the prompt nitrovasodilation of *S*-nitrosocaptopril (Fig. 1, bottom panel). The dose-dependent hypotensive effect of *S*-nitrosocaptopril (0.125, 1.25 and 12.5 mg/kg, i.v.) is illustrated in Fig. 2, which persisted for 14 ± 3 min (0.125 mg/kg), 23 ± 3 min (1.25 mg/kg), 48 ± 8 min (12.5 mg/kg), respectively. Captopril did not produce significant acute hypotensive effects even at high concentrations (12.5 mg/kg). Fig. 3 illustrates changes in glomerular filtration rate in response to *S*-nitrosocaptopril in these awake rats during the observation period. Glomerular filtration rate fell significantly only at 20 min after a bolus injection of high dose of *S*-nitrosocaptopril (12.5 mg/kg, $P < 0.01$), followed by an transient overshoot above normal levels. The basal urine flow in the awake rats was 22.2 ± 3.4 μl/min. Administration of *S*-nitrosocaptopril (i.v.) caused concomitant decreases in urine flow to 18.4 ± 4.5 μl/min (0.125 mg/kg, $P > 0.05$), 14.0 ± 2.4 μl/min (1.25 mg/kg, $P < 0.05$), and 7.5 ± 2.6 μl/min (12.5 mg/kg, $P < 0.01$ vs. basal urine flow), respectively. However, within 1 h after administration of *S*-nitrosocaptopril (12.5 mg/kg), glomerular filtration rate and urine flow returned toward control values. It is important to mention that the transient decreases in glomerular filtration rate and urine flow are primarily the consequence of markedly acute reduction by 53.9% in mean arterial pressure induced by *S*-nitrosocaptopril (12.5 mg/kg, Figs. 1 and 2). By comparison, captopril had little effect on mean arterial pressure, glomerular filtration rate, and urine flow in these awake rats even at doses of 12.5 mg/kg during the 2 h observation period (Fig. 3).

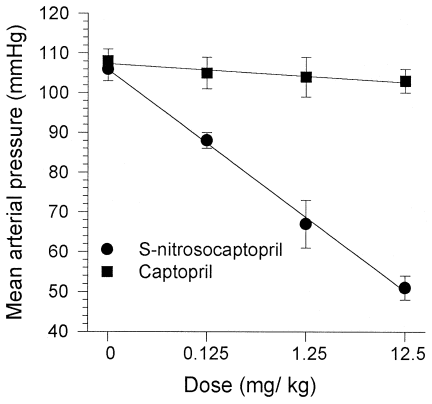

Fig. 2. Comparison of acute effects of *S*-nitrosocaptopril and captopril on mean arterial pressure in chronically-instrumented awake rats. The compounds were given by bolus i.v.. Each point represents the mean ± S.E. ($n = 5$).

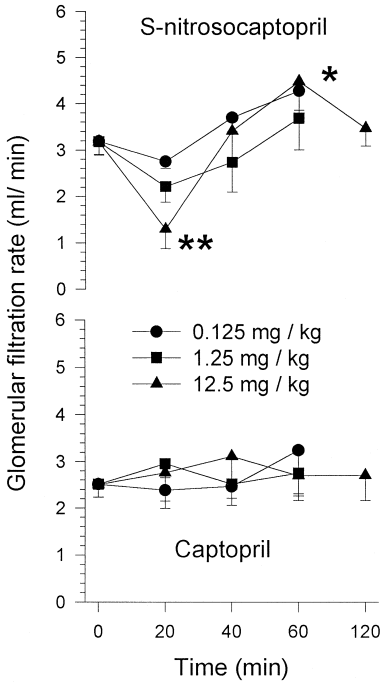

Fig. 3. Time course of changes in glomerular filtration rate in chronically-catheterized awake rats after bonus i.v. *S*-nitrosocaptopril (top) or captopril (bottom). Results are presented as the mean ± S.E.. Statistical difference from basal value at time zero: *, $P < 0.05$; **, $P < 0.01$, $n = 5$–6.

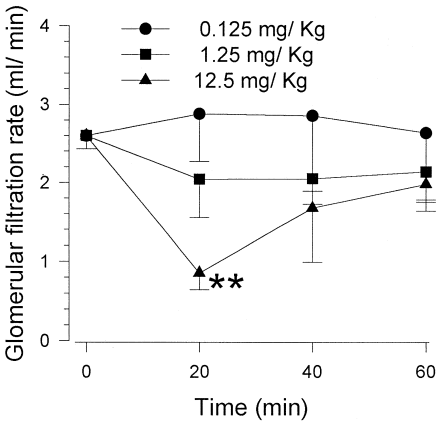

Fig. 4. Time-course of changes in glomerular filtration rate in anesthetized Wistar rats after bolus i.v. *S*-nitrosocaptopril. Results are presented as the mean ± S.E.. Statistical difference from basal value at time zero: $**$, $P < 0.01$, $n = 5–6$.

3.3. Effects of intravenous doses of S-nitrosocaptopril on blood pressure and glomerular filtration rate in anesthetized rats

Injection of *S*-nitrosocaptopril at doses of 0.125, 1.25 and 12.5 mg/kg caused a dose-dependent reduction in mean arterial pressure. The blood pressure fell immediately after injection. At doses of 0.125 and 1.25 mg/kg, *S*-nitrosocaptopril decreased mean arterial pressure by 29.5 and 37.5%, respectively, while having no significant effect upon glomerular filtration rate. *S*-nitrosocaptopril at a dose of 12.5 mg/kg reduced blood pressure by 50.1%, and glomerular filtration rate and urine flow decreased significantly by 69.2 and 60.3% at 20 min after *S*-nitrosocaptopril administration. The reduction in glomerular filtration rate and urine flow was transient. Glomerular filtration rate and urine flow returned to the basal levels prior to recovery of blood pressure (Fig. 4).

To clearly distinguish mechanisms of action of *S*-nitrosocaptopril from captopril, the antagonism between NO synthase inhibitor L-NMMA and *S*-nitrosocaptopril or captopril was examined in the anesthetized Wistar rats. Pretreatment of the rats with L-NMMA (30 mg/kg, dissolved in 0.9% NaCl, i.v.) gradually but significantly increased mean arterial pressure from 102 ± 7 to 131 ± 14 mmHg ($P < 0.01$) within 30 min, and decreased heart rates from 396 ± 47 to 366 ± 19 beats/min ($P > 0.05$). When the steady hypertensive state was reached, either *S*-nitrosocaptopril or captopril was administrated (0.125 mg/kg, i.v.). *S*-nitrosocaptopril significantly antagonized L-NMMA effects and reduced mean arterial pressure from 131 ± 14 to 103 ± 11 mmHg ($P < 0.01$, $n = 4$). In contrast, captopril did not diminished the hypertension induced by L-NMMA (Fig. 5). This result suggests the difference in acute mechanisms between the two compounds, and that only *S*-nitrosocaptopril, as an exogenous NO supplement, alleviates L-NMMA hypertension.

3.4. Effects of oral doses of S-nitrosocaptopril on two-kidney one-clipped Goldblatt hypertensive rats

Awake systolic blood pressure prior to initiation of antihypertensive treatment did not differ among the three groups of clipped rats. Some 10 days after treatment with *S*-nitrosocaptopril (25 mg/kg, b.i.d.) systolic blood pressure was significantly decreased from 151 ± 2 to 120 ± 4 mmHg ($n = 6$, $P < 0.01$) comparable to that in normotensive Wistar control rats (114 ± 2 mmHg, $P > 0.05$;

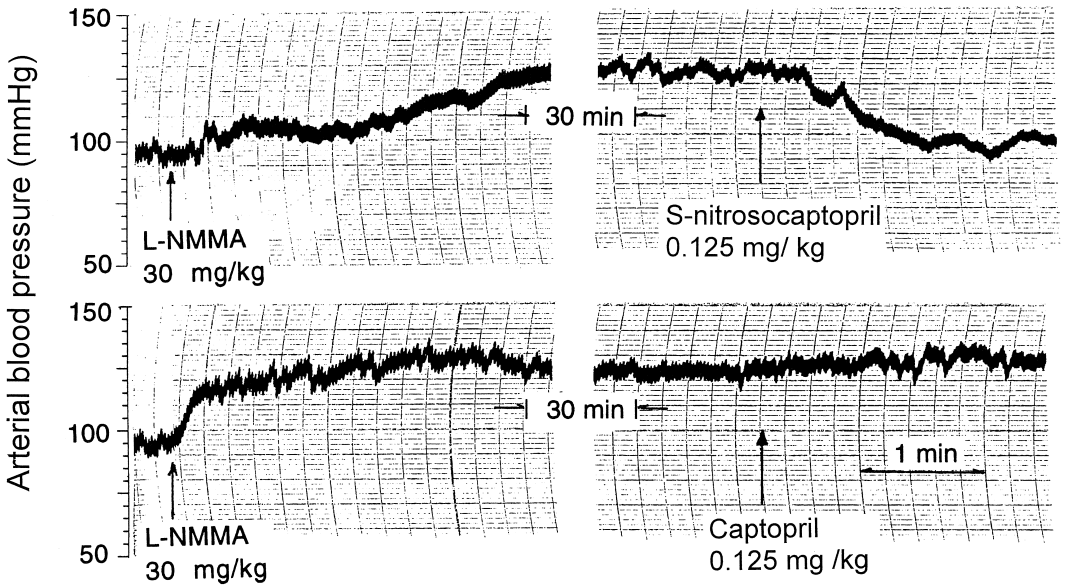

Fig. 5. Reversal effects of *S*-nitrosocaptopril on hypertension induced by i.v. N^G-monomethyl-L-arginine (L-NMMA) in anesthetized rats. In contrast, captopril did not antagonize the L-NMMA-induced hypertension. Tracings represent four experiments.

Fig. 6). Treatment of two-kidney, one-clipped Goldblatt hypertensive rats with captopril (25 mg/kg, b.i.d.) progressively caused a decrease in systolic blood pressure from 154 ± 5 to 135 ± 8 mmHg as the duration of treatment with captopril increased ($P < 0.05$). However, a significant difference in systolic blood pressure between normotensive Wistar rats and captopril-treated two-kidney, one-clipped Goldblatt hypertensive rats was demonstrated ($P < 0.05$). In addition, the decrease in systolic blood pressure was significantly greater with *S*-nitrosocaptopril than with captopril ($P < 0.05$, analysis of variance), suggesting that captopril may not be as potent as *S*-nitrosocaptopril in treating two-kidney, one-clipped Goldblatt hypertensive rats (Fig. 6).

Ten days after the treatment was initiated, clearance studies were performed on these two-kidney, one-clipped Goldblatt hypertensive rats under anesthesia. In *S*-nitrosocaptopril- and captopril-treated two-kidney, one-clipped Goldblatt hypertensive rats, glomerular filtration rate was obviously reduced, whereas in two-kidney, one-clipped Goldblatt hypertensive rats, glomerular filtration rate was higher (Fig. 7), which was likely due to significant increases in both glomerular capillary hydrostatic pressure and glomerular hydrostatic pressure gradient (De Nicola et al., 1990). The hematocrit, however, was not appreciably affected either by the induction of hypertension or by the two different treatments. At the end of clearance experiments, the rats were euthanized and both kidneys were removed and weighed. There were no significant differences in body weight between these groups of rats; however, the nonclipped left kidneys were significantly larger and the clipped right kidneys were significantly smaller than control kidneys from normal rats. The wet kidney weights (g) in normal rats was 1.51 ± 0.11 (left), and 1.51 ± 0.13 (right); in two-kidney, one-clipped Goldblatt hypertensive rats, 0.74 ± 0.32 (left clipped), and 2.04 ± 0.19 (right); in *S*-nitrosocaptopril-treated two-kidney, one-clipped Goldblatt hypertensive rats, 0.76 ± 0.21(left clipped), and 1.90 ± 0.16 (right); in captopril-treated two-kidney, one-clipped Goldblatt hypertensive rats, 0.68 ± 0.17 (left clipped), and 1.93 ± 0.10 (right). The increase in renal mass in the nonclipped kidney is primarily the consequence of compensatory hypertrophy due to the decreased renal mass in the clipped kidney (Huang et al., 1981).

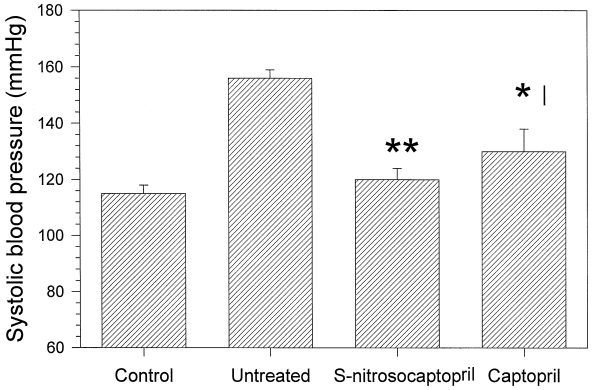

Fig. 6. Comparison of effects of *S*-nitrosocaptopril and captopril on blood pressure in two-kidney, one-clipped Goldblatt hypertensive rats. Oral doses of *S*-nitrosocaptopril and captopril were administrated 25 mg/kg by gavage (b.i.d) for 10 days after hypertension. *S*-nitrosocaptopril normalized blood pressure and exhibited more potent effects than captopril did. Whereas, captopril reduced, but did not normalize blood pressure. Each data represents the mean ± S.E. of six rats. * and * * denote $P < 0.05$ and < 0.01 vs. untreated two-kidney, one-clipped Goldblatt hypertensive rats, respectively. | denotes $P < 0.05$ vs. normotensive Wistar control.

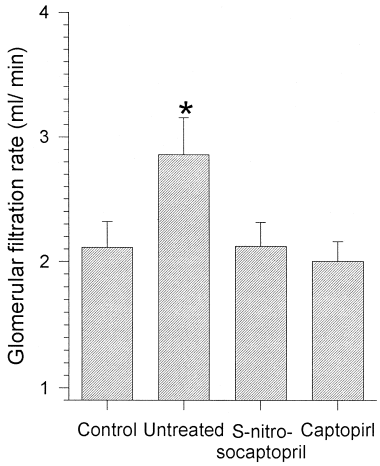

Fig. 7. Comparison of effects of *S*-nitrosocaptopril and captopril on glomerular filtration rate in two-kidney one-clipped Goldblatt hypertensive rats. Oral doses of *S*-nitrosocaptopril and captopril were administrated 25 mg/kg by gavage (b.i.d) for 10 days after hypertension. The glomerular filtration rate data for each rat were calculated using average values of three clearance periods of 20-min after equilibration post surgery. Each data represents the mean ± S.E. of seven rats. * * denotes $P < 0.01$ vs. normotensive control.

4. Discussion

The most striking finding from this study was that *S*-nitrosocaptopril, when orally administered, produced potent hypotensive effects in awake normotensive (Fig. 1) and two-kidney, one-clipped Goldblatt hypertensive rats (Fig. 6). In contrast, captopril did not produce any acute hypotensive effect in normotensive rats, and the hypotensive effect of captopril was significantly less than *S*-nitrosocaptopril in two-kidney, one-clipped Goldblatt hypertensive rats (Fig. 6). The acute oral hypotensive effect can be only contributed to NO cleaved and released from the compound because the parent compound captopril at the same dose (50 mg/kg) did not produce appreciate acute hypotensive effect. The failure to observe the hypotensive effect of captopril is consistent with experiments conducted by Takanohashi et al. (1996) showing that captopril (40 mg/kg, p.o.) did not cause the significant

hypotensive effect in the deoxycorticosterone acetate-salt hypertensive rats. Previous studies have demonstrated that *S*-nitrosocaptopril could directly relax the in vitro blood vessels (Loscalzo et al., 1989; Lin et al., 1998). *S*-nitrosocaptopril, when intravenously administrated, effectively reduced canine blood pressure, attenuated the pressor response to angiotensin I (Shaffer et al., 1991), and increased epicardial coronary diameter and coronary blood flow (Nakae et al., 1995). In the present study, we showed that the magnitude and duration of hemodynamic response correlate well with the graded increases in either oral (Fig. 1) or intravenous doses (Fig. 2). Taking account of these facts, we concluded that the oral effect by *S*-nitrosocaptopril observed is indicative of a direct absorption of this compound into the circulation, after which *S*-nitrosocaptopril exhibits its activity in target organs. The mechanism by which *S*-nitrosocaptopril is absorbed and distributed so quickly and exhibits activity immediately, is as yet unidentified, but may involve the same absorption manner as captopril does, the latter readily crosses the biological membranes of the gastrointestinal tract (Migdalof et al., 1984). However, strong evidence for the absorption, excretion and pharmacokinetical profile of *S*-nitrosocaptopril are under further investigation.

The data presented herein demonstrated acute hypotensive effects of *S*-nitrosocaptopril in both awake and anesthetized rats when intravenously administrated. The hypotensive effect of intravenous *S*-nitrosocaptopril appears to be more potent in anesthetized rats than that in awake rats. This is because anesthetization results in impairment of central and peripheral nervous reflex as well as hormonal mechanisms by which the sympathetic vasoconstrictor system regulates pressure rapidly. In our earlier studies we showed that *S*-nitrosocaptopril produced dose-dependent vasorelaxation in isolated rabbit aorta with endothelium either intact or denuded (Lin et al., 1998), this endothelium-independent relaxation indicates a direct release and supplement of the relaxing factor NO from *S*-nitrosocaptopril to vascular smooth muscle, regardless of whether the endothelial NO synthase activity is impaired or not. A more direct study of the ability of *S*-nitrosocaptopril to reverse the hypertension produced by a NO synthase inhibitor was afforded by the in vivo antagonism experiments (Fig. 5), in which the systemic NO production was inhibited, and only *S*-nitrosocaptopril could immediately reverse the hypertension secondary to the endothelial NO synthase inhibition, by supplementing the exogenous NO to the target tissues. In view of the effective good reversal by *S*-nitrosocaptopril of hypertension induced by L-NMMA, it is somewhat unexpected that this NO donor at the concentration of 0.125 mg/kg, about 240-fold lower than that of L-NMMA (30 mg/kg), could almost completely counteract the L-NMMA-induced hypertension in the case of NO synthase inhibition. In contrast, captopril failed to provide any detectable reversal of L-NMMA-induced hypertension. It has been shown that in anesthetized rats pretreated with angiotensin receptor antagonist losartan or angiotensin converting enzyme inhibitor enalaprilat, NO synthase inhibitor L-NAME still significantly increased systemic blood pressure (Sigmon et al., 1992). The hypertensive response to NO synthase inhibition has also been shown to be reduced by angiotensin converting enzyme inhibition, angiotensin AT1 receptor antagonists (Rajagopalan and Harrison, 1996). Therefore, the hypertensive response to NO synthase inhibitors is mediated by more than the inhibition of endothelium-derived NO. The cardiovascular system possesses receptors to many of the same agonists that activate NO release from the endothelium. Thus, the ultimate effect of any given stimulus or inhibition for NO release is a balance between the vasodilator effect of NO vs. the direct vasoconstrictive effect of the stimulus on the vascular smooth muscle. The specific potent antagonism of *S*-nitrosocaptopril against the NO synthase inhibitor L-NMMA strongly support that the mechanism by which *S*-nitrosocaptopril has an acute blood pressure-lowering effect is governed by its action as an NO donor. This is also in agreement with reports that L-NMMA pretreatment attenuated the hypotensive effect of captopril in spontaneous hypertensive rats, but it did not impair the hypotensive effect of sodium nitroprusside (Cachofeiro et al., 1992). *S*-nitrosocaptopril produced an acute decrease in blood pressure upon bolus intravenous administration similar to nitroglycerin (Nakae et al., 1995). The vasoactivities of *S*-nitrosocaptopril appear to be reflected on chemical structures of *S*-nitrosocaptopril as well as its velocities of NO liberation. Indeed, the substitution at the SH position with NO group produced a significant influence on the vasoactivity of *S*-nitrosocaptopril.

It is of great interest to note that modest doses of *S*-nitrosocaptopril, as an exogenous NO donor, do not significantly affect glomerular filtration rate and urine flow, and transient changes in glomerular filtration rate and urine flow only occur after a major decrease in blood pressure produced by a large intravenous dose of *S*-nitrosocaptopril. In awake rats, for example, glomerular filtration rate begins to decrease (Fig. 3) only when there is a reduction by $\sim$50% in blood pressure (Fig. 2) induced by high doses of *S*-nitrosocaptopril (12.5 mg/kg); in anesthetized rats, glomerular filtration rate significantly decreased (Fig. 4) only when mean arterial pressure was reduced by 50.1% by *S*-nitrosocaptopril (12.5 mg/kg). We also found that a regional infusion of *S*-nitrosocaptopril (10–1000 nM) into the rat renal artery produced a transient and reversible reduction in renal perfusion pressure in a dose dependent manner (Lin et al., 1998), indicating a direct renal vasorelaxing effect of *S*-nitrosocaptopril. The renal effect produced by high systemic doses of *S*-nitrosocaptopril is presumably due to major decreases in aortic pressure perfusing the kidney, which in turn leads to decreases in plasma flow and hydraulic pressures in the glomerular capillary. The ability of the kidney to maintain renal blood flow and glomerular filtration rate constant

over a wide range of perfusion pressures is the well-recognized renal autoregulation, to which several factors contribute. Indeed, the glomerular filtration rate values rapidly returned to the control level prior to the recovery of blood pressure after a bolus injection of *S*-nitrosocaptopril (12.5 mg/kg, Fig. 3). Taken together, these results suggest preservation of efficiency of glomerular filtration rate autoregulation by *S*-nitrosocaptopril, and that *S*-nitrosocaptopril is a renal-sparing antihypertensive agent. It has been reported that the profound systemic hypotension induced by NO produced by *Escherichia coli* endotoxin is accompanied by relatively good preservation of renal perfusion (Shultz et al., 1990), indicating that NO may exert a renoprotective effect. This hypothesis is further supported by studies showing that, after induced production of NO, rats did not develop renal functional impairment or significant glomerular injury (Shultz and Raij, 1992). Despite the fact that decreases in blood pressure after systemic administration of *S*-nitrosocaptopril (12.5 mg/kg) caused the transient decreases in glomerular filtration rate and urine flow, a reduction in arteriolar resistance can, in turn, increase the hydraulic pressure thereby raising the gradient favoring filtration and resulting in an overshoot of glomerular filtration rate above normal values (Fig. 3).

Since this is the first report to show the acute oral bioavailability of *S*-nitrosocaptopril, we further exploited whether the compound exerts a therapeutic effect in two-kidney, one-clipped Goldblatt hypertensive rats. In the two-kidney, one-clipped Goldblatt hypertensive rats, the hypertension is a result of increased renin activity due to the decreased flow/pressure in the clipped kidney (Sigmon and Beierwaltes, 1993). The plasma angiotensin II level and blood pressure are elevated after clipping (De Nicola et al., 1990). NO synthesis inhibition also results in more potent increases in blood pressure and vascular resistance in the two-kidney, one-clipped Goldblatt hypertensive rats than in normotensive control (Sigmon and Beierwaltes, 1993). Several factors might be responsible for the development of two-kidney, one-clipped Goldblatt hypertensive rats, which includes reduced NO production, a reduced ability to vasodilate arterioles, and/or imbalance between the L-arginine: NO pathway and the increased endogenous angiotensin II. It is well documented that NO directly counteracts the vasoconstrictive influences of angiotensin II and other vasoconstrictors that angiotensin converting enzyme inhibitors may not directly antagonize. It is tempting to simultaneously use both an exogenous NO donor to restore the impaired L-arginine: NO pathway, and an angiotensin converting enzyme inhibitor to block production of angiotensin II in the two-kidney, one-clipped Goldblatt hypertensive rats. Indeed, as shown here (Fig. 6), *S*-nitrosocaptopril caused significantly reductions in blood pressure and glomerular filtration rate resulting in normalization of pressure and renal function after 10 days of oral dosing. Captopril reduced blood pressure to a level lower than that of two-kidney, one-clipped Goldblatt hypertensive rats, but not to the level of normotensive rats. The results suggest that *S*-nitrosocaptopril exerts an additive effect of an exogenous NO donor and an angiotensin converting enzyme inhibitor on the two-kidney, one-clipped Goldblatt hypertensive rats. However, chronic oral administration of *S*-nitrosocaptopril or captopril did not appreciably prevent the reductions in mass in clipped kidneys. The potent antihypertensive effect of *S*-nitrosocaptopril in the two-kidney, one-clipped Goldblatt hypertensive rats might be explained through several mechanisms. First, the difference between *S*-nitrosocaptopril and captopril on blood pressure and renal function might only be attributable to the NO moiety in the structure of *S*-nitrosocaptopril, which may share the same mechanism of action as that of endogenous endothelium-derived NO, and be able to supply or replace the impaired L-arginine: NO pathway. Second, *S*-nitrosylation of captopril confers the properties of a NO donor but does not lose its activity against angiotensin converting enzyme (Loscalzo et al., 1989; Sigmon and Beierwaltes, 1993). Third, it has been demonstrated that both captopril and its metabolites such as captopril disulfide, captopril–cysteine, captopril–glutathione play a role in the treatment of hypertension (Kripalani et al., 1980; Huang et al., 1981). Therefore, a plausible involvement of captopril and respective captopril disulfide derived from the homolytic cleavage of the S–N bond of *S*-nitrosocaptopril in the chronic treatment of two-kidney, one-clipped Goldblatt hypertensive rats cannot be excluded. Very importantly, the therapeutic properties of captopril metabolites confer *S*-nitrosocaptopril to be the most advantageous of the known NO donor drugs with less toxicity.

It is generally believed that human hypertension is caused by a defect in endothelial NO synthesis or by an impaired vascular response to endothelium-derived NO (Benjamin and Vane, 1996). The elimination of intrinsic NO-mediated vasodilation allows endogenous vasoconstrictors such as angiotensin II to predominate, therefore, the loss of an NO effect in various conditions could contribute to the process of cardiovascular disease, which includes abnormal vasoconstriction and hypertension, platelet aggregation and thrombus formation, vascular proliferation and atherosclerosis. In addition, on the basis of these considerations, there has been increasing scientific and public interest in the development of an efficient NO donor. *S*-nitrosocaptopril, categorized as a combination of an NO donor and an angiotensin converting enzyme inhibitor (Loscalzo et al., 1989; Park, 1992), could fulfil the demand.

In summary, the results demonstrate that *S*-nitrosocaptopril is a potent antihypertensive agent which preferentially decreases blood pressure. This compound possesses oral pharmacological activities, and most of its acute effects may ultimately be mediated by NO. The tissue specificity of the parent captopril and *S*-nitrosocaptopril metabolites may be also related to the therapeutic properties of the compound. *S*-nitrosocaptopril provides the ad-

vantage of both acute and chronic effective anti-hypertensive therapy.

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