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The Effects of S-Nitrosocaptopril on Canine Coronary Circulation[1]

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ABSTRACT

Nitroglycerin (NTG) has been used for more than a century for the relief of angina pectoris and is regarded as an extrinsic donor of nitric oxide (NO). Captopril, an angiotensin-converting enzyme inhibitor (ACEI), is reported to have beneficial effects on survival in acute myocardial infarction. In this study, a hybrid compound of NO and ACEI, S-nitrosocaptopril (S-NO-Cap), was synthesized, which was characterized by its molecular structure. Its coronary effects in chronically instrumented dogs were compared with those of NTG and captopril. S-NO-Cap (50 μg/kg i.v.) increased epicardial coronary diameter (CoD) by a maximum of 5.2% for more than 20 min. NTG (15 μg/kg i.v.) increased CoD by a maximum of 4.6% for 10 min, *i.e.*, the effect was equipotent with that of S-NO-Cap (50 μg/kg i.v.). However, S-NO-Cap produced its maximal CoD increase more slowly than NTG did. Both S-NO-Cap and NTG transiently increased coronary blood flow. However, NTG had a more potent effect than S-NO-Cap (28.4 *vs.* 40.8 ml/min, respectively). Captopril, on the other hand, had almost no effect on either CoD or coronary blood flow. Thus, the vasodilatory action of S-NO-Cap more closely resembled that of NTG than that of captopril. Therefore, S-NO-Cap may dilate coronary arteries by virtue of its NO moiety rather than by its ACEI properties. These findings indicate that S-NO-Cap is a potential antianginal drug.

NTG has been used for more than a century for the treatment of angina pectoris and is regarded as a donor of NO (Feelisch and Noack, 1987; Benjamin *et al.*, 1991), which is considered by some to be endothelium-derived relaxing factor (Ignarro *et al.*, 1987; Palmer *et al.*, 1987). More recently, it has been suggested that endothelium-derived relaxing factor is more likely to be an S-nitrosothiol, such as S-nitrosocysteine, rather than NO (Myers *et al.*, 1990). Fujioka *et al.* (1993) reported that thiol-containing compounds have direct vasorelaxant effects on dog coronary arteries. Thus, the formation of S-nitrosothiols is a possible mechanism for the vasorelaxant effects of nitrates (Ignarro *et al.*, 1981). The depletion of tissue thiols by nitrates is a critical factor in the development of tolerance to the vasodilatory actions of these compounds (Needleman and Johnson, 1973). In addition, several studies have reported that thiol donors prevent or reverse nitrate tolerance (Torresi *et al.*, 1985; May *et al.*, 1987).

Captopril is a thiol-containing ACEI that has been reported to have beneficial effects in patients with ischemic heart disease (Pfeffer *et al.*, 1992), in those with heart failure (Captopril Multicenter Research Group, 1983) and in those with insulin resistance (Pollare *et al.*, 1989). Some studies have shown that captopril improves the patient's quality of life (Testa *et al.*, 1993). Captopril reportedly reverses NTG tolerance because of its thiol moiety (Lawson *et al.*, 1991) and reacts with NO^+ to form S-NO-Cap, a hybrid compound of ACEI and NO (Loscalzo *et al.*, 1989; Park, 1992).

Loscalzo *et al.* (1989) and Cooke *et al.* (1989) showed that S-NO-Cap dilated isolated bovine coronary and femoral arteries and significantly increased cyclic GMP levels. They also demonstrated that S-NO-Cap and captopril had similar potencies as ACEIs (Loscalzo *et al.*, 1989).

To clarify the mechanisms of action of S-NO-Cap, we compared the hemodynamic effects of this agent with those of NTG and captopril on systemic blood pressure and coronary hemodynamics in chronically instrumented dogs.

Materials and Methods

Materials

Captopril was obtained from Sankyo (Tokyo, Japan). *t*-Butyl nitrite (Aldrich Chemical, Tokyo, Japan), acetonitrile (Kanto Chemical, Tokyo, Japan), chloroform (Nakarai Tesque, Kyoto, Japan), dicyclohexylamine (Sigma, St. Louis, MO), NTG (25 mg of NTG with

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ABBREVIATIONS: ACEI, angiotensin-converting enzyme inhibitor; AP, arterial blood pressure; CBF, coronary blood flow; CoD, coronary artery diameter; CVR, coronary vascular resistance; HR, heart rate; mAP, mean arterial blood pressure; NO, nitric oxide; NTG, nitroglycerin; S-NO-Alb, S-nitrosoalbumin; S-NO-Cap, S-nitrosocaptopril; LSI/MS, liquid secondary ion mass spectrometry analysis.

2535 mg of D-mannitol in 50 ml of distilled water, Nippon Kayaku, Tokyo, Japan) and sodium pentobarbital (Dainabot, Osaka, Japan) were also used. All other reagents were of the highest grade available.

S-NO-Cap was synthesized according to the method of Shaffer *et al.* (1991). In brief, captopril (220 mg) in 10 ml of chloroform was cooled on ice and protected from light. Drops of *t*-butyl nitrite (0.6 ml) were added until the chloroform solution of captopril turned red. The solution was then concentrated under reduced pressure. The resulting red oil was dissolved in 6 ml of cold acetonitrile and residual solid material was removed by filtration through filter paper. Dicyclohexylamine (0.2 ml) was added to the filtrate and the product was allowed to crystallize for at least 2 hr at −20°C. The resulting pink solid was rinsed with cold acetonitrile and dried under reduced pressure to give S-NO-Cap. The presence of this chemical was confirmed by an increase in ultraviolet absorption at 335 nm in a methanol solution of the products.

The structure of S-NO-Cap was determined by LSI/MS analysis using a VG 70−4SE (VG Analytical, Manchester, UK). The intensity of $[M+H]^+$ ions (m/z 247, corresponding to S-NO-Cap) was clearly observed in the LSI/MS spectrum with 3-nitrobenzyl alcohol as a matrix. On the other hand, the intensity of $[M-H]^-$ ions (m/z 245, corresponding to S-NO-Cap) was observed in the negative LSI/MS spectrum when either glycerol or 3-nitrobenzyl alcohol was used as a matrix. As we previously reported (Amano *et al.*, 1994), fragment ions of S-NO-Cap revealed the structure of S-NO-Cap by LSI tandem mass spectrometry at m/z 247 and m/z 245, which corresponded to the $[M+H]^+$ and $[M-H]^-$ ions of S-NO-Cap, respectively.

Effects of S-NO-Cap, NTG and Captopril on Coronary Vasculature in Chronically Instrumented Dogs

Animals were treated according to the guidelines for animal experimentation prepared by the Japanese Association for Laboratory Animal Science. Adult mongrel dogs of either sex weighing 11 to 20 kg were anesthetized with an i.v. injection of sodium pentobarbital (30 mg/kg). The dogs were mechanically ventilated with room air mixed with oxygen using Harvard respirators (Harvard Apparatus, South Natick, MA). Under aseptic conditions, we performed a thoracotomy in the fifth intercostal space and suspended the heart in a pericardial cradle. Two ultrasonic crystals (vessel diameter CVD 2300, Sonotek, San Diego, CA) were implanted, facing each other, in the left circumflex coronary artery, approximately 3 cm from its orifice, to measure CoD. A Doppler flow probe was implanted distal to the ultrasonic crystals on the same artery to measure CBF (pulsed Doppler PD-20, model VF-1, Crystal Biotech, Holliston, MA). Phasic changes in CoD were measured continuously with an ultrasonic dimension gauge (Goodman and Castellana, 1982) that produced a voltage that was linearly proportional to the transit time of acoustic impulses traveling between the opposing ultrasonic crystals at 1.54×10^6 mm/sec. The voltage drift of the instrument's output was virtually zero for 3 hr. To ensure the reliability of the measurement, the received ultrasonic signal was continuously monitored on an oscilloscope. Any major changes in the triggering level of the received signal invalidated the experiment. Experiments were conducted 7 to 30 days after the surgical procedure.

Experimental Protocol

Crystals of S-NO-Cap were dissolved in saline to a final volume of 2 ml just before use. NTG solution was diluted with saline to produce a final volume of 2 ml. Captopril powder was dissolved in saline to a volume of 2 ml just before use.

On the day of measurement, the dogs were anesthetized with sodium pentobarbital and supplemental doses of pentobarbital were administered as necessary (approximately 20 mg/kg in total). The left circumflex CoD and CBF were measured as previously described (Hashimoto *et al.*, 1991) and recorded continuously on an eight-channel recorder (model RCM 3000, Nihon Koden, Tokyo, Japan). Femoral AP was measured with a water-filled pressure transducer. HR was measured by an HR counter (AT-600G, Nihon Koden, Tokyo, Japan) that was triggered by an AP pulse.

Protocol 1: effects of S-NO-Cap, NTG and captopril on coronary arteries of chronically instrumented dogs. After CoD, CBF, AP and HR had stabilized for more than 30 min, S-NO-Cap (50 μg/kg), NTG (15 μg/kg) or captopril (50 μg/kg) was administered in an i.v. bolus in randomly selected groups ($n = 7$). The interval between drug administrations was more than 1 hr; during this time, all hemodynamic parameters returned to the control levels.

Protocol 2: dose-response curves of the action of S-NO-Cap and captopril on dog coronary arteries. S-NO-Cap ($n = 6$) at doses of 6.25, 12.5, 25, 50 and 100 μg/kg was administered as a series of i.v. bolus injections. On different experimental days, captopril ($n = 6$) at doses of 6.25, 12.5, 25, 50 and 100 μg/kg was administered as a series of i.v. bolus injections in the same dogs. Each drug was administered only after the parameters had returned to the control levels.

Statistical Analysis

All data are expressed as the mean value ± S.E.M. The Doppler shift measured with the coronary flow velocity probe in kilohertz was converted to blood flow with the equation: $q = 1.25 \times d^2 \times f$, where q is blood flow (in milliliters per minute), d is vessel inner diameter (in millimeters) and f is the Doppler shift (in kilohertz). In the present study, d was approximated as the inner diameter of the flow probe ($d = 2$ mm). The CVR was calculated from the mean systemic blood pressure (in millimeters of mercury) and the mean CBF (in milliliters per minute). Differences from control values were analyzed by Student's t test for paired data. Peak responses to S-NO-Cap and NTG were compared using Student's t test for unpaired data. Responses to S-NO-Cap and captopril were compared by an analysis of variance for repeated measures. A P value of less than 0.05 was considered statistically significant. Calculations were performed with the Statview II and Excel 4.0 (Microsoft Japan, Tokyo, Japan) programs on a Macintosh computer (Apple Computer Japan, Tokyo, Japan).

Results

Hemodynamic effects of S-NO-Cap, NTG and captopril. The effects of S-NO-Cap, NTG and captopril on HR, femoral AP, CoD and CBF were examined in chronically instrumented closed-chest dogs. A typical example of the effects of these agents is shown in figure 1.

We obtained hemodynamic data from seven dogs to calculate mean values ± S.E.M., as shown in figure 2 and table 1. S-NO-Cap increased HR from 127.9 ± 16.0 to 162.1 ± 13.9 beats/min ($n = 7$, $P < .001$, by Student's paired t test) 2 min after administration, a 30.2% increase from base line. This significant increase in HR induced by S-NO-Cap persisted for more than 20 min. On the other hand, although NTG increased HR by 57.7%, from 120.1 ± 14.9 to 179.3 ± 8.4 beats/min ($n = 7$, $P < .001$, by Student's paired t test) within 1 min after injection, HR returned to 125.3 ± 14.4 beats/min within 5 min, which was not significantly different from the control level. Captopril slightly increased HR (by 8.5%) from 120.6 ± 15.9 to 129.4 ± 15.5 beats/min ($n = 7$, $P < .001$) 2 min after administration and the level remained slightly, but significantly, elevated for more than 10 min after injection. S-NO-Cap reduced mAP by a maximum of 12.7%, from 97.4 ± 4.2 to 85.4 ± 5.4 mm Hg ($P < .001$), within 1 min after injection. The mAP remained significantly depressed for more than 5 min. NTG reduced the mAP from 97.0 ± 4.2 mm Hg by a maximum of 38.7% just after injection but the level

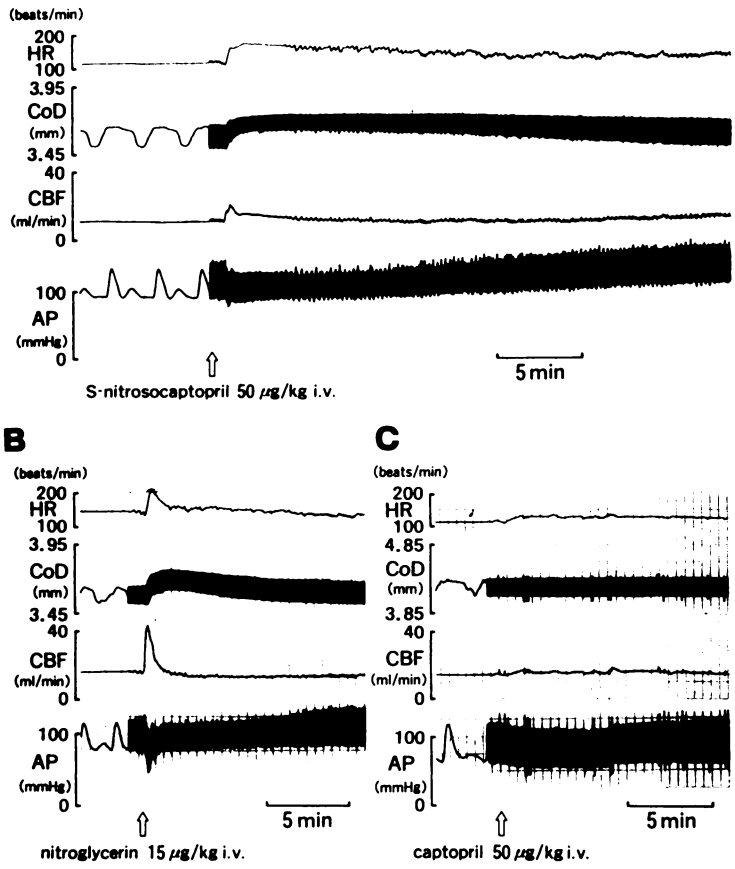

Fig. 1. Effects of i.v. S-NO-Cap (50 μg/kg; A), NTG (15 μg/kg; B) and captopril (50 μg/kg; C) on HR, phasic circumflex CoD, mean CBF and femoral AP. S-NO-Cap produced an increase in CoD for more than 20 min, whereas it increased CBF for only a short period of time. NTG also increased CoD for more than 10 min, whereas it increased CBF only transiently. On the other hand, captopril had almost no effect on CoD or CBF.

returned to near-control levels within 2 min after administration. Captopril reduced mAP from 94.7 ± 5.8 mm Hg by a maximum of 11.1% 5 min after injection and this value remained significantly depressed for more than 5 min.

Coronary effects of S-NO-Cap (50 μg/kg) compared with those of NTG (15 μg/kg) or captopril (50 μg/kg). The effects of S-NO-Cap, NTG and captopril on CoD, CBF and CVR are shown in figures 1 and 2 and table 1. CoD rose similarity with both S-NO-Cap and NTG but remained elevated significantly longer with S-NO-Cap than with NTG (> 20 min *vs.* 10 min). S-NO-Cap increased CoD by a maximum of 5.2% 5 min after administration and NTG increased CoD by a maximum of 4.6% 1 min after administration. This difference was not significant (by Student's unpaired *t* test). In contrast, captopril did not significantly increase CoD.

The maximal increase in CBF was greater ($P < .01$, by Student's unpaired *t* test) with NTG (142.6% from 17.1 ± 1.0 ml/min) than with S-NO-Cap (72.0% from 17.5 ± 2.1 ml/min). However, there was no difference between these two drugs in their peak reduction of CVR (NTG, −51.2%; S-NO-Cap, −47.2%). NTG induced both its maximal increase in CBF and its maximal decrease in CVR immediately after injection but these values quickly returned to control levels soon thereafter. S-NO-Cap also produced both its maximal increase in CBF and its maximal decrease in CVR just after administration and these values returned to control levels within 2 min after treatment. On the other hand, although captopril increased CBF and decreased CVR only slightly, these effects persisted for more than 5 min (fig. 2, B and C).

Hemodynamic and coronary effects of i.v. S-NO-Cap and captopril at doses of 6.25, 12.5, 25, 50 and 100 μg/kg. A typical example of the effects of S-NO-Cap on CoD and CBF is shown in figure 3 and the percent changes in HR, mAP, mean CoD, mean CBF and CVR brought about by S-NO-Cap and captopril are shown in figure 4. S-NO-Cap (6.25–100 μg/kg) produced dose-dependent decreases in mAP. Similar doses of captopril also produced dose-dependent decreases in mAP. However, the decrease in mAP was greater with S-NO-Cap than with captopril ($n = 6$, $P < .001$). There was no significant difference between the effects of the two drugs on HR, although the increase in HR with S-NO-Cap tended to be greater than that with captopril. At doses of 6.25 to 100 μg/kg, S-NO-Cap increased CoD in a dose-dependent manner. In contrast, captopril produced no significant change in CoD at these doses. S-NO-Cap at 6.25 μg/kg initiated a significant increase in CBF and also produced a significant reduction in CVR. At doses of 6.25 to 100 μg/kg, S-NO-Cap increased CBF and decreased CVR in a dose-dependent manner. In this dose range, the increase in CBF was significantly greater with S-NO-Cap than with captopril ($n = 6$, $P < .01$). The decrease in CVR with S-NO-Cap was also significantly greater than that with captopril ($n = 6$, $P < .001$).

Discussion

The major finding in this study was that S-NO-Cap had NO-like effects on dog coronary arteries. Previous reports

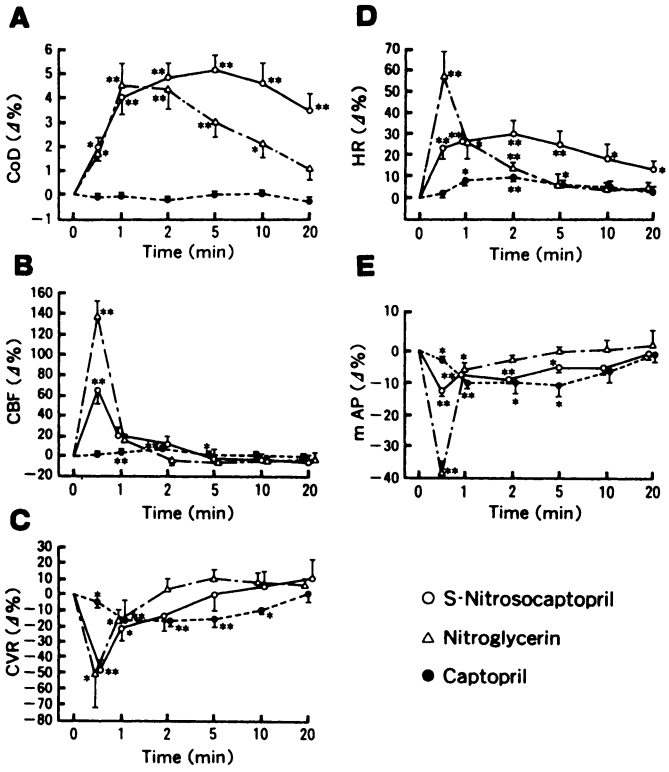

Fig. 2. Time course of percent changes in mean CoD (A), CBF (B), CVR (C), HR (D) and mAP (E) after i.v. injections of S-NO-Cap (○, 50 μg/kg), NTG (△, 15 μg/kg) and captopril (●, 50 μg/kg). Each point represents the mean ± S.E.M. of seven experiments. Asterisks indicate significant differences from the value at time zero: *P < .05; **P < .01.

(Loscalzo *et al.*, 1989; Park, 1992) claimed that S-NO-Cap has both an NO-like action and ACEI activity. S-NO-Cap has been reported to affect isolated bovine arteries (Loscalzo *et al.*, 1989), systemic hemodynamics in dogs (Shaffer *et al.*, 1991) and human platelets (Stamler *et al.*, 1992b; Amano *et al.*, 1994). The vasodilatory effects of S-NO-Cap on isolated arterial strips are reportedly due to its nitrate-like action (Loscalzo *et al.*, 1989; Cooke *et al.*, 1989) because captopril has no vasodilatory effect. The systemic effects of S-NO-Cap have been attributed to both ACEI and nitrate action (Shaffer *et al.*, 1991). Finally, the antiplatelet effects of this agent were demonstrated to be due mainly to an NO-like action, with little ACEI action being involved (Amano *et al.*, 1994).

In this study, we compared the effects of S-NO-Cap with those of NTG on canine coronary circulation to clarify whether an NO-like action was involved in S-NO-Cap-induced coronary arterial relaxation. S-NO-Cap dilated epicardial coronary arteries persistently but increased CBF only transiently. At the same time, AP was reduced and HR was increased. NTG dilated coronary arteries as persistently and potently as S-NO-Cap and also increased CBF only transiently. The hypotensive effects of NTG were transient, with reflex tachycardia being manifested. We compared the effects of captopril and S-NO-Cap on the coronary vasculature to determine whether any ACEI activity was involved. Captopril had almost no effect on CoD or CBF but did have a hypotensive effect with reflex tachycardia. Thus, the vasodilatory action of S-NO-Cap more closely resembled that of NTG than that of captopril. These results indicate that the coronary vasorelaxing effects of S-NO-Cap are due to the presence of an NO moiety rather than to any ACEI activity. The mechanism by which S-NO-Cap, a nitrosothiol, dilates coronary arteries is believed to involve 1) its action as an NO donor (Feelisch and Noack, 1987) or 2) the stabilized NO moiety it contains (Amano *et al.*, 1994). We previously examined the release of NO from S-NO-Cap in plasma (Amano *et al.*, 1994) and Krebs-Henseleit buffer solution. No release of NO was detected in plasma (Amano *et al.*, 1994) but some release was found in Krebs-Henseleit buffer (unpublished observation). Thus, the biological activity of S-NO-Cap may be due to the S-nitrosothiol moiety itself rather than its decomposition to NO. Further studies are needed to determine whether S-NO-Cap is an NO donor *in vivo*. In addition, the activation of soluble guanylate cyclase reportedly represents signal transduction for both S-NO-Cap and NTG because methylene blue has been shown to inhibit the vasodilatory actions of both compounds (Cooke *et al.*, 1989).

Increases in CoD induced by S-NO-Cap (50 μg/kg) were more gradual and long lasting (> 20 min) than those induced by NTG (15 μg/kg), although the peak dilatory effects of S-NO-Cap (50 μg/kg) and NTG (15 μg/kg) on large coronary arteries were similar. The difference in the duration of epicardial coronary dilatation is not related to any secondary effects, such as flow-dependent coronary vasodilatation, because the increases in CBF induced by these two agents were only transient (within 1 min). Moreover, the maximal increase in CBF was less ($P < .01$) with S-NO-Cap (50 μg/kg; 72% from 17.5 ml/min) than with NTG (15 μg/kg; 143% from 17.1 ml/min). In addition, an *in vitro* study has shown that both S-NO-Cap and NTG induce direct endothelium-independent smooth muscle relaxation (Loscalzo *et al.*, 1989). NTG is metabolized to NO and mono- or di-NTG by glutathione-S-transferase (Kurz *et al.*, 1993) and NO is rapidly oxidized to nitrite and nitrate anions. Thus, the difference in the time taken to dilate epicardial coronary arteries may be related to differences in the metabolism of the two drugs *in vivo*.

S-NO-Cap increased CBF and decreased CVR for only a short time, as shown in figure 2 and table 1; these changes reflect dilatation of small-resistance coronary vessels. NTG also transiently increased CBF and decreased CVR. Higher doses of S-NO-Cap and NTG were required to dilate resistant coronary arteries compared with those required to dilate large coronary arteries. On the other hand, although captopril only slightly increased CBF and slightly decreased CVR, these effects were relatively long lasting. If the pharmacological effects of S-NO-Cap are due mainly to NO rather than to ACEI, as has been previously reported (Loscalzo *et al.*, 1989; Cooke *et al.*, 1989; Shaffer *et al.*, 1991; Park, 1992), the effect of S-NO-Cap on coronary resistance vessels could be attributed mainly to an NO-like action, with little involvement of ACEI activity. This idea is supported by the results of our preliminary experiments, in which we found that intracoronary arterial injection of captopril (30–300 μg) had no effect on CBF or systemic hemodynamics in open-chest anesthetized dogs (data not shown). Thus, it would appear that the increase in CBF induced by captopril in the present study was modified by reflex mechanisms secondary to the decrease in systemic blood pressure. The vasodilatory effects of S-NO-Cap on coronary resistance vessels also appear to be due to the NO-like action of this compound.

In the present study, S-NO-Cap, NTG and captopril all reduced AP with reflex tachycardia. The duration of the

TABLE 1. Hemodynamic and coronary effects of S-No-Cap (50 μg/kg), NTG (15 μg/kg) and captopril (50 μg/kg). All values represent mean ± S.E.M. [a]P < .05; [b]P < .01; [c]P < .001 *vs.* control.

	Control	< 1min	1min	2min	5min	10min	20min
Heart rate (beats/min)							
S-Nitrosocaptopril (n=7)	127.9± 16.0	152.7±13.1[b]	158.3±14.9[c]	162.1±13.9[c]	156.9± 15.4[b]	149.1±15.4[a]	143.3± 18.3[a]
Nitroglycerin (n=7)	120.1± 14.9	179.3± 8.4[c]	147.1±14.5[b]	133.3±14.5[b]	125.3± 14.4	122.1±14.2	124.7± 13.9
Captopril (n=7)	120.6± 15.9	122.4±16.3	128.3±15.6[b]	129.4±15.5[c]	127.6± 16.5[b]	125.9±16.8[a]	122.3± 16.1
Mean arterial blood pressure (mmHg)							
S-Nitrosocaptopril (n=7)	97.4± 4.2	85.4± 5.4[c]	89.3± 3.1[b]	88.5± 2.7[b]	92.0± 3.1[a]	92.2± 2.1	96.3± 2.8
Nitroglycerin (n=7)	97.0± 4.2	59.5± 4.3[c]	91.4± 4.6[a]	94.9± 3.9	97.3± 4.7	98.9± 5.4	100.2± 5.7
Captopril (n=7)	94.7± 5.8	91.1± 5.6[a]	85.6± 5.5[a]	84.7± 6.2[a]	84.5± 6.6[a]	88.1± 5.7	93.2± 5.2
Mean coronary blood flow (ml/min)							
S-Nitrosocaptopril (n=7)	17.5± 2.1	28.4± 1.7[c]	20.4± 1.5	18.5± 1.8	16.5± 1.5	15.9± 1.6	15.7± 1.7
Nitroglycerin (n=7)	17.1± 1.0	40.8± 2.0[c]	19.5± 1.2	16.2± 0.9	15.6± 1.1	16.2± 1.2	17.1± 1.4
Captopril (n=7)	15.3± 0.7	15.8± 0.7	16.6± 0.8[c]	16.5± 1.0[b]	16.0± 0.9[a]	15.8± 0.9	15.4± 0.8
Coronary vascular resistance (mmHg/ml/min)							
S-Nitrosocaptopril (n=7)	6.10±0.70	3.08±0.29[b]	4.51±0.30[a]	5.00±0.41	5.82±0.47	6.14±0.57	6.53±0.65
Nitroglycerin (n=7)	5.85±0.51	1.50±0.17[c]	4.82±0.39[a]	5.96±0.39	6.43±0.53	6.36±0.62	6.14±0.63
Captopril (n=7)	6.30±0.53	5.84±0.45[a]	5.25±0.42[b]	5.26±0.51[b]	5.41±0.53[a]	5.70±0.48[a]	6.20±0.56
Coronary diameter (mm)							
S-Nitrosocaptopril (n=7)	3.39±0.18	3.46±0.18[a]	3.52±0.17[c]	3.55±0.17[c]	3.57±0.18[c]	3.55±0.18[b]	3.51±0.18[b]
Nitroglycerin (n=7)	3.40±0.18	3.45±0.18[a]	3.55±0.17[b]	3.54±0.17[b]	3.50±0.18[b]	3.46±0.18[a]	3.44±0.18
Captopril (n=7)	3.39±0.12	3.39±0.12	3.39±0.12	3.38±0.12	3.39±0.12	3.39±0.12	3.38±0.11

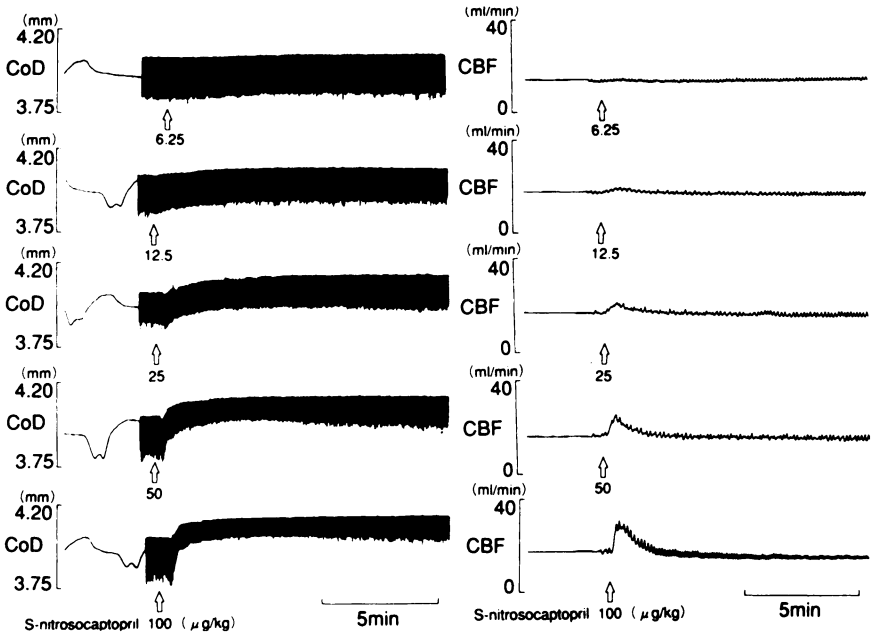

Fig. 3. Effects of bolus injections of i.v. S-NO-Cap at doses of 6.25, 12.5, 25, 50 and 100 μg/kg on circumflex CoD and mean CBF in representative dogs.

hypotensive effects of S-NO-Cap and captopril were similar; the hypotensive effects of NTG were of short duration. Coronary vasorelaxation was specific for S-NO-Cap but not for captopril. Thus, the systemic vasorelaxing effects of ACEI cannot explain S-NO-Cap-induced coronary arterial relaxation.

NTG has been regarded as the most effective agent for the relief of angina pectoris. Angina pectoris generally results from fixed stenosis and/or vasospasm in one or more of the large coronary arteries. Therefore, the ability of NTG to dilate large coronary arteries could explain its beneficial effect and its ability to reduce preload and afterload (Frishman, 1985). In contrast, the dilation of small coronary arteries results in a coronary steal phenomenon and leads to the exacerbation of ischemia. As we have shown, the vasodilatory effects of S-NO-Cap were greater in proximal large coronary arteries than in distal small coronary arteries, which is similar to the effects of NTG (Chu *et al.*, 1987, 1990; Hashimoto *et al.*, 1991). Moreover, S-NO-Cap produced a longer lasting vasodilation in large coronary arteries than NTG; it had a lesser effect on coronary blood flow than NTG. These results suggest that S-NO-Cap may have antianginal effects through

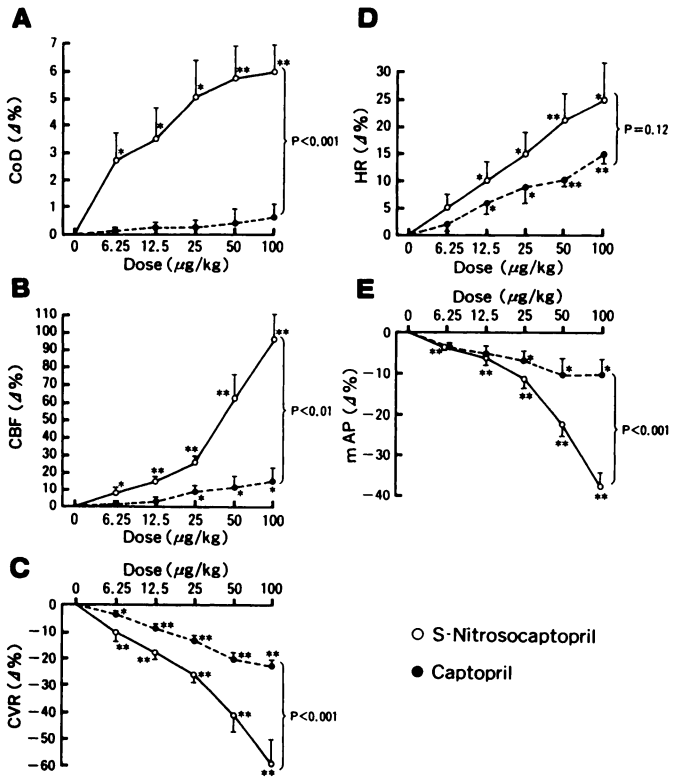

Fig. 4. Percent changes in mean CoD (A), CBF (B), CVR (C), HR (D) and mAP (E) induced by bolus injections of i.v. S-NO-Cap (○) and captopril (●) at doses of 6.25, 12.5, 25, 50 and 100 μg/kg in anesthetized dogs. Each point represents the mean ± S.E.M. of six experiments. Asterisks indicate significant differences from the control: *P < .05; **P < .01. The brackets indicate significant differences between the responses to the two drugs.

its vasodilatory effect on large coronary arteries because of its NO-like action.

Zhang *et al.* (1993) reported that another S-nitrosothiol, S-NO-Alb (Stamler *et al.*, 1992a,b) had vasodilatory effects on coronary arteries in chronically instrumented dogs. They found that S-NO-Alb produced a longer lasting vasodilation in large coronary arteries than NTG and the time to peak vasodilation was much greater with S-NO-Alb. Furthermore, the sensitivity of coronary resistance vessels to S-NO-Alb was markedly less than their sensitivity to NTG. Thus, the vasodilatory effects of S-NO-Cap on coronary vessels *in vivo* more closely resemble those of S-NO-Alb rather than NTG. These properties of S-nitrosylated derivatives may offer greater therapeutic advantages than NTG in the treatment of ischemic heart disease.

As stated earlier, S-NO-Cap has also been reported to exert antiplatelet effects (Loscalzo *et al.*, 1989; Stamler *et al.*, 1992b; Amano *et al.*, 1994). S-NO-Cap has been shown to inhibit ADP-induced platelet aggregation in a dose-dependent manner, with an IC_{50} of 2.5 μM. This effect is equivalent to those of sodium nitroprusside and S-NO-Alb (Takahashi *et al.*, 1994). These effects of S-NO-Cap may be beneficial in the treatment of ischemic heart disease.

Captopril has been reported to have beneficial effects on survival after acute myocardial infarction (Pfeffer *et al.*, 1992) and has been shown to potentiate isosorbide dinitrate-induced coronary dilatation (van Gilst *et al.*, 1987; Metelitsa *et al.*, 1992). In anesthetized dogs, the hypotensive effect of NTG was potentiated by pretreatment with captopril but not with S-NO-Cap (Shaffer *et al.*, 1991). Although, there is currently not enough evidence to show that the coadministration of nitrates and captopril leads to the production of S-NO-Cap in humans, such production of S-NO-Cap could conceivably be related to the beneficial effects of captopril (Shaffer *et al.*, 1991).

The biological effects of S-nitrosothiols may be due, at least in part, to the S-nitrosylation of thiols within enzymes, receptors and/or ion channels, as reported by Fujioka *et al.* (1993), instead of the action of a specific S-nitrosothiol, such as captopril.

In summary, S-NO-Cap exerted a vasodilatory effect on canine coronary arteries *in vivo*, which was essentially related to its actions as a nitrate. These findings suggest that S-NO-Cap is a potential antianginal drug that also possesses ACEI activity.

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References

Amano, M., Takahashi, M., Kosaka, T. and Kinoshita, M.: Differential inhibition of platelet aggregation and calcium mobilization by nitroglycerin and stabilized nitric oxide. J. Cardiovasc. Pharmacol. **24:** 860–866, 1994.

Benjamin, N., Dutton, J. A. E. and Ritter, J. M.: Human vascular smooth muscle cells inhibit platelet aggregation when incubated with glyceryl trinitrate: evidence for generation of nitric oxide. Br. J. Pharmacol. **102:** 847–850, 1991.

Captopril Multicenter Research Group: A placebo-controlled trial of captopril in refractory chronic congestive heart failure. J. Am. Coll. Cardiol. **2:** 755–763, 1983.

Chu, A. and Cobb, F. R.: Effects of atrial natriuretic peptide on proximal epicardial coronary arteries and coronary blood flow in conscious dogs. Circ. Res. **61:** 485–491, 1987.

Chu, A., Murray, J. J., Lin, C.-C., Russel, M., Hagen, P.-O. and Cobb, F. R.: Preferential proximal coronary dilation by activators of guanylate cyclase in awake dogs. Am. J. Physiol. **259:** H340–H345, 1990.

Cooke, J. P., Andon, N. and Loscalzo, J.: S-nitrosocaptopril. II. Effects on vascular reactivity. J. Pharmacol. Exp. Ther. **249:** 730–734, 1989.

Feelisch, M. and Noack, E. A.: Correlation between nitric oxide formation during degradation of organic nitrates and activation of guanylate cyclase. Eur. J. Pharmacol. **139:** 19–30, 1987.

Frishman, W. H.: Pharmacology of the nitrates in angina pectoris. Am. J. Cardiol. **56:** 8I–13I, 1985.

Fujioka, H., Horiike, K., Takahashi, M., Ishida, T., Kinoshita, M. and Nozaki, M.: Triphasic vascular effects of thiol compounds and their oxidized forms on dog coronary arteries. Experientia **49:** 47–50, 1993.

Goodman, C. A. and Castellana, F. S.: A digital sonomicrometer for two-point length and velocity measurements. Am. J. Physiol. **243:** H634–H639, 1982.

Hashimoto, K., Kinoshita, M. and Ohbayashi, Y.: Coronary effects of nicorandil in comparison with nitroglycerin in chronic conscious dogs. Cardiovasc. Drugs. Ther. **5:** 131–138, 1991.

Ignarro, L. J., Buga, G. M., Wood, K. S., Byrns, R. E. and Chaudhuri, G.: Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc. Natl. Acad. Sci. U. S. A. **84:** 9265–9269, 1987.

Ignarro, L. J., Lippton, H., Edwards, J. C., Baricos, W. H., Hyman, A. L., Kadowitz, P. J. and Gruetter, C. A.: Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: Evidence for the involvement of S-nitrosothiols as active intermediates. J. Pharmacol. Exp. Ther. **218:** 739–749, 1981.

Kurz, M. A., Boyer, T. D., Whalen, R., Peterson, T. E. and Harrison, D. G.: Nitroglycerin metabolism in vascular tissue: Role of glutathione S-transferases and relationship between NO and NO_2^- formation. Biochem. J. **292:** 545–550, 1993.

Lawson, D. L., Nichols, W. W., Mehta, P. and Mehta, J. L.: Captopril-induced reversal of nitroglycerin tolerance: Role of sulfhydryl group vs. ACE-inhibitory activity. J. Cardiovasc. Pharmacol. **17:** 411–418, 1991.

Loscalzo, J., Smick, D., Andon, N. and Cooke, J.: S-nitrosocaptopril. I. Molecular characterization and effects on the vasculature and on platelets. J. Pharmacol. Exp. Ther. **249:** 726–729, 1989.

May, D. C., Popma, J. J., Black, W. H., Schaefer, S., Lee, H. R., Levine, B. D. and Hillis, L. D.: *In vivo* induction and reversal of nitroglycerin tolerance in human coronary arteries. N. Engl. J. Med. **317:** 805–809, 1987.

METELITSA, V. I., MARTESEVICH, S. Y., KOZYREVA, M. P. AND SLASTNIKOVA, I. D.: Enhancement of the efficacy of isosorbide dinitrate by captopril in stable angina pectoris. Am. J. Cardiol. **69:** 291–296, 1992.

MYERS, P. R., MINOR, R. L., JR., GUERRA, R., JR., BATES, J. N. AND HARRISON, D. G.: Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. Nature (Lond.) **345:** 161–163, 1990.

NEEDLEMAN, P. AND JOHNSON, E. M., JR.: Mechanism of tolerance development to organic nitrates. J. Pharmacol. Exp. Ther. **184:** 709–715, 1973.

PALMER, R. M. J., FERRIGE, A. G. AND MONCADA, S.: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature (Lond.) **327:** 524–526, 1987.

PARK, J.-W.: Dual role of S-nitrosocaptopril as an inhibitor of angiotensin-converting enzyme and a nitroso group carrier. Biochem. Biophys. Res. Commun. **189:** 206–210, 1992.

PFEFFER, M. A., BRAUNWALD, E., MOYÉ, L. A., BASTA, L., BROWN, E. J., JR., CUDDY, T. E., DAVIS, B. R., GELTMAN, E. M., GOLDMAN, S., FLAKER, G. C., KLEIN, M., LAMAS, G. A., PACKER, M., ROULEAU. J., ROULEAU, J. L., RUTHERFORD, J., WERTHEIMER, J. H. AND HAWKINS, C. M., ON BEHALF OF THE SAVE INVESTIGATORS: Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction: Results of the survival and ventricular enlargement trial. N. Engl. J. Med. **327:** 669–677, 1992.

POLLARE, T., LITHELL, H. AND BERNE, C.: A comparison of the effects of hydrochlorothiazide and captopril on glucose and lipid metabolism in patients with hypertension. N. Engl. J. Med. **321:** 868–873, 1989.

SHAFFER, J. E., LEE, F., THOMSON, S., HAN, B.-J., COOKE, J. P. AND LOSCALZO, J.: The hemodynamic effects of S-nitrosocaptopril in anesthetized dogs. J. Pharmacol. Exp. Ther. **256:** 704–709, 1991.

STAMLER, J. S., JARAKI, O., OSBORNE, J., SIMON, D. I., KEANEY, J., VITA, J., SINGEL, D., VALERI, C. R. AND LOSCALZO, J.: Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. Proc. Natl. Acad. Sci. U. S. A. **89:** 7674–7677, 1992a.

STAMLER, J. S., SIMON, D. I., OSBORNE, J. A., MULLINS, M. E., JARAKI, O., MICHEL, T., SINGEL, D. J. AND LOSCALZO, J.: S-nitrosylation of proteins with nitric oxide: Synthesis and characterization of biologically active compounds. Proc. Natl. Acad. Sci. U. S. A. **89:** 444–448, 1992b.

TAKAHASHI, M., NAKAE, I., AMANO, M. AND KINOSHITA, M.: Anti-anginal effects of S-nitroso-captopril to coronary artery and on platelets (Abstract). J. Am. Coll. Cardiol. **23:** 211A, 1994.

TESTA, M. A., ANDERSON, R. B., NACKLEY, J. F., HOLLENBERG, N. K. AND THE QUALITY-OF-LIFE HYPERTENSION STUDY GROUP: Quality of life and antihypertensive therapy in men. A comparison of captopril with enalapril. N. Engl. J. Med. **328:** 907–913, 1993.

TORRESI, J., HOROWITZ, J. D. AND DUSTING, G. J.: Prevention and reversal of tolerance to nitroglycerine with N-acetylcysteine. J. Cardiovasc. Pharmacol. **7:** 777–783, 1985.

VAN GILST, W. H., DE GRAEFF, P. A., SHOLTENS, E., DE LANGEN, C. D. J. AND WESSELING, H.: Potentiation of isosorbide dinitrate-induced coronary dilatation by captopril. J. Cardiovasc. Pharmacol. **9:** 254–255, 1987.

ZHANG, J., SOMERS, M., GOMES, E., GASTON, B. J. AND COBB, F. R.: Effects of S-nitroso-albumin and nitroglycerin on coronary conductance and flow regulatory vessels (Abstract). Circulation. **88:** I-34, 1993.

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