

ORIGINAL ARTICLE

The effect of *S*-nitrosocaptopril and *S*-nitroso-*N*-acetyl-*D,L*-penicillamine on blood glucose concentration and haemodynamic parameters

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Summary

Studies have shown that *S*-nitrosothiols (RSNOs) are able to affect glucose metabolism and blood pressure in animal models. This paper describes an investigation into the effect of two RSNOs, *S*-nitrosocaptopril (CapSNO) and *S*-nitroso-*N*-acetyl-*D,L*-penicillamine (SNAP) on fasting and postprandial blood glucose concentration, and systolic and diastolic blood pressures. Rats administered intravenously with CapSNO and SNAP, using dosages of 2.0, 5.0 and 12.5 mg/kg BW, showed a dose-dependent hyperglycaemic effect. Intravenous administration of 12.5 mg/kg BW of CapSNO and SNAP caused a statistically significant increase in fasting blood glucose concentration compared to rats treated with the same dosage of captopril. SNAP-treated rats showed a significantly greater elevation of fasting (F2) blood glucose concentration (5.91 ± 0.27 mmol/l) compared to CapSNO-treated rats (5.11 ± 0.08 mmol/l). However there was no significant difference in postprandial blood glucose concentrations. SNAP, CapSNO and captopril significantly reduced both systolic and diastolic blood pressures. This was accompanied by an increase in heart rate. The anti-hypertensive property of CapSNO and SNAP was more significant than that of captopril. CapSNO was more potent than SNAP in reducing blood pressure, suggesting that CapSNO may act via a combined mechanism that involves ACE inhibition and NO release.

Key words: *S*-nitrosothiols; *S*-nitroso-*N*-acetylpenicillamine; *S*-nitrosocaptopril; hyperglycaemia; haemodynamic parameters

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INTRODUCTION

S-nitrosothiols (RSNOs) are formed from a reaction between the sulfhydryl functional group of thiols, and nitric oxide (NO), nitrogen dioxide (NO₂) or nitrous acid (HNO₂) (Tsikas et al. 1999). They have the general formula R-S-N=O where R represents a hydrocarbon chain and S-N=O the thionitrite group which is responsible for the chemical properties of the RSNOs. There are studies which dispute the formation

of *in vivo* RSNOs via nitrosylation of a thiol because once NO is in the vascular system, it is readily mopped up by haemoglobin thus reducing the concentration of available NO (Moro et al. 1995, Singh et al. 1996a). However, a nitrosylation reaction does take place *in vivo*. The inhibition (Mohr et al. 1996) and activation (Salehi et al. 1996) of certain key enzymes and proteins occur as a result of NO nitrosating the thiol group or groups present at the active site, or attached to, these molecules.

The biological effects of RSNO mimic the ubiquitous NO as this occurs because RSNOs can readily decompose to yield NO. Transnitrosation is believed to be the mechanism by which RSNOs decompose *in vivo*. The mechanism involves a nucleophilic attack by the thiolate anion (RS⁻) on the negatively charged nitrogen atom of the S-nitroso group (Matthew and Kerr 1993). Reductants such as ascorbic acid (Aqart and Dasgupta 2004), copper ions (Askew et al. 1995) and thiols such as glutathione and cysteine (Singh et al. 1996b) are able to increase the rate of decomposition of RSNOs. Nitric oxide and RSNOs share similar physiological characteristics: they possess anti-inflammatory properties (Keeble and Moore 2002), are potent vasodilators (Ignarro et al. 1981), and they inhibit the aggregation of platelets (Langford et al. 1994).

S-nitrosocaptopril (CapSNO) and S-nitroso-N-acetyl-D,L-penicillamine (SNAP) are examples of synthetic RSNOs with great therapeutic potential. S-nitrosocaptopril, which is formed via the nitrosylation of captopril, has a primary structure and is more stable *in vitro* than SNAP, which has a tertiary structure (Aqart and Dasgupta 2004). Captopril is a thiol that is able to inhibit the activity of the angiotensin converting enzyme (ACE) thus causing vasodilation. Nitrosylation of this compound to form CapSNO does not interfere with the inhibition properties of ACE (Loscalzo et al. 1989) and as a result this hybrid molecule has both thionitrite and ACE inhibition moieties which confer vasodilation.

S-nitrosothiols are also able to cause a significant reduction in blood pressure and are considered potent vasodilators (Cooke et al. 1989, Shaffer et al. 1991). They are fast replacing organic nitrates as they avoid the side effects associated with the use of these compounds (Megson 2000).

S-nitrosothiols reduce blood pressure by a mechanism that involves a NO/sGC pathway (Craven and DeRubertis 1983, Moynihan and Roberts 1994). Decomposition of the RSNOs liberates NO, which is able to diffuse to neighboring target cells where it acts primarily through the activation of sGC to generate cGMP from GTP, thus bringing about a response through a reduction in intracellular calcium levels

(Ignarro et al. 1999). One limitation to the therapeutic use of RSNOs is the possible hyperglycaemic effect that this group of molecules elicits. It is reported that RSNOs are able to increase blood glucose concentration via the release of NO (McGrowder et al. 1999), even though these compounds have been shown to be therapeutically effective prior to decomposition (Nakae et al. 1995). CapSNO has been found to dilate coronary arteries by virtue of its NO moiety and is a potential anti-anginal drug (Nakae et al. 1995).

This study compared the effects of CapSNO and SNAP with captopril, on fasting and postprandial blood glucose concentration, and haemodynamic parameters such as systolic and diastolic blood pressures. The study sought to deduce whether captopril or its RSNO derivative, CapSNO is more potent in causing a significant reduction in blood pressure and/or affecting blood glucose concentration in normoglycaemic rats.

MATERIALS AND METHODS

Animals

Rats were obtained from the Basic Medical Sciences Animal House, University of the West Indies, Mona. Healthy male and female Wistar mixed breed rats were used within the weight range of 250–350 grams. The rats were kept in separate cages according to their sex to eliminate the possibility of impregnation. The rats were fed a daily diet of Purina Lab Chow and water administered *ad libitum*. All procedures were approved by and conducted in accordance with the guidelines of The University of the West Indies Animal Care and Use Committee.

Sample preparation

A dosage of 12.5 mg/kg body weight (BW) of captopril, CapSNO, SNAP was used for the analysis. Saline (0.4 ml, 0.9% NaCl) was used to dissolve captopril and CapSNO, and dimethyl sulfoxide [(DMSO; 0.4 ml, 50%); Sigma Chemical Co, St. Louis, USA] was used to dissolve SNAP just before the beginning of the analysis. The solution was then administered into the tail vein of the rat immediately after the first blood sample was obtained for analysis.

Oral glucose tolerance test (OGTT)

Oral glucose tolerance (OGTT) was carried out using an automated method. The glucometer (Miles Inc. Diagnostics Division, Indiana, USA) was calibrated with standard solutions before use to allow for

optimum performance. The test was carried out on rats to determine the effect of captopril, SNAP (Sigma Chemical Co., St. Louis, USA) and CapSNO via intravenous administration on blood glucose levels in normoglycaemic rats. S-nitroso-captopril was synthesized as previously described (Nakae et al. 1995). Each compound was administered at a dosage of 12.5 mg/kg BW. Rats were fasted for approximately fifteen (15) hours; during this time only water was given *ad libitum*. A fasting blood sample at time (F1, 0 min) was obtained from the rat's tail, immediately after which the drug was administered via the intravenous route. Fasting blood samples were taken at the 0.5 hour interval (F2, 30 min), and for a further 1 hour. Immediately after the 1 hour (0 h, 60 min) a fasting sample was taken, and a glucose load at a dosage of 1.75 g/kg BW was then administered orally, after which postprandial blood samples were taken at 0.5 hour intervals for a further 2.5 hours.

Haemodynamic studies

Blood pressure was non-invasively measured by determining the tail blood volume with a volume pressure recording sensor and an occlusion tail-cuff (CODA System, Kent Scientific, Torrington, USA) (Euser and Cipolla 2007). This system utilizes two cuffs, i.e., the occlusion cuff, which first inflates and then prevents blood flow to the vein and the VPR sensor cuff which measures the return of blood flow by measuring the swelling cause by arterial pulsations from the blood flow.

Systolic blood pressure (SBP) was measured at the first appearance of the tail swelling, and diastolic blood pressure (DBP) was automatically calculated when the swelling ceased. Cuff inflation was carried out repetitively and automatically to produce the most consistent and accurate blood pressure data. The rats were acclimatized for 15 minutes before baseline readings were obtained (-5, -10 min). After the 10 min baseline analysis, a dosage of 12.5 mg/kg BW of the compound or control was administered (via i.v.) intravenously. Haemodynamic data were obtained at 5 min intervals for a further 55 min. The control group was treated with saline solution (0.4 ml 0.9%).

Statistics

Each data point was expressed as mean \pm s.e.m. For OGTT a minimum sample size of 9 rats was used to carry out the investigation. Blood samples were taken in duplicates for each time interval. For the haemodynamic studies, a total of 4 rats were used for each compound analyzed. At each time interval, 15 readings were taken and the average determined. In addition each experiment was carried out three times.

The significance between compounds was determined using the Student's t-test or two way analysis of variance (ANOVA) at the significance level $2\alpha=0,05$.

RESULTS

The effect of CapSNO, SNAP and captopril on blood glucose concentration

A dose-dependent hyperglycaemic response was observed when CapSNO, SNAP or captopril was administered intravenously at dosages of 2.5, 5.0 and 12.5 mg/kg BW. The hyperglycaemic effect was most significant in rats treated with 12.5 mg/kg BW of CapSNO or SNAP as these rats showed sustained elevation of the blood glucose concentration throughout the duration of the experiment.

S-nitrosocaptopril and SNAP caused a significant increase in the blood glucose concentration when compared with captopril-treated rats (Fig. 1). During the fasting stage of the experiment CapSNO-treated rats showed a statistically significant increase in the blood glucose concentration from 3.83 ± 0.10 mmol/l (F1, 0 min) to 5.11 ± 0.08 mmol/l (F2, 30 min). However, SNAP-treated rats showed a more significant increase in the fasting blood glucose concentration during this time interval, i.e. from 3.81 ± 0.09 mmol/l to 5.91 ± 0.27 mmol/l. After the glucose load was administered, CapSNO-treated rats showed a gradual decrease in blood glucose concentration from a maximum of 6.81 ± 0.23 mmol/l at the 0.5 h (90 min) interval to 5.46 ± 0.12 mmol/l at the 2.5 h (210 min) interval. SNAP-treated rats also showed a decrease in the blood glucose concentration from a maximum of 6.44 ± 0.24 mmol/l at 1 h (120 min) interval to 5.26 ± 0.05 mmol/l at the 2.5 h (210 min) interval. Even though SNAP-treated rats showed a 0.5 h delay in response to the glucose load when compared with the rats that were administered CapSNO, the postprandial data did not reveal any significant difference between the two groups.

Both CapSNO and SNAP caused a more significant increase in the fasting and postprandial blood glucose concentrations than captopril. The fasting data for the latter group showed that there was an increase in the blood glucose concentration from 3.50 ± 0.11 mmol/l at the F1 (0 min) interval to 4.14 ± 0.11 mmol/l at the F2 (30 min) interval. After the glucose load was given orally the blood glucose concentration continued to increase to a maximum of 6.08 ± 0.16 mmol/l at the 1.0 h (120 min) postprandial interval. There was a significant decrease thereafter to 4.63 ± 0.12 mmol/l to the 2.5 h (210 min) interval. Therefore rats treated with 12.5 mg/kg BW of

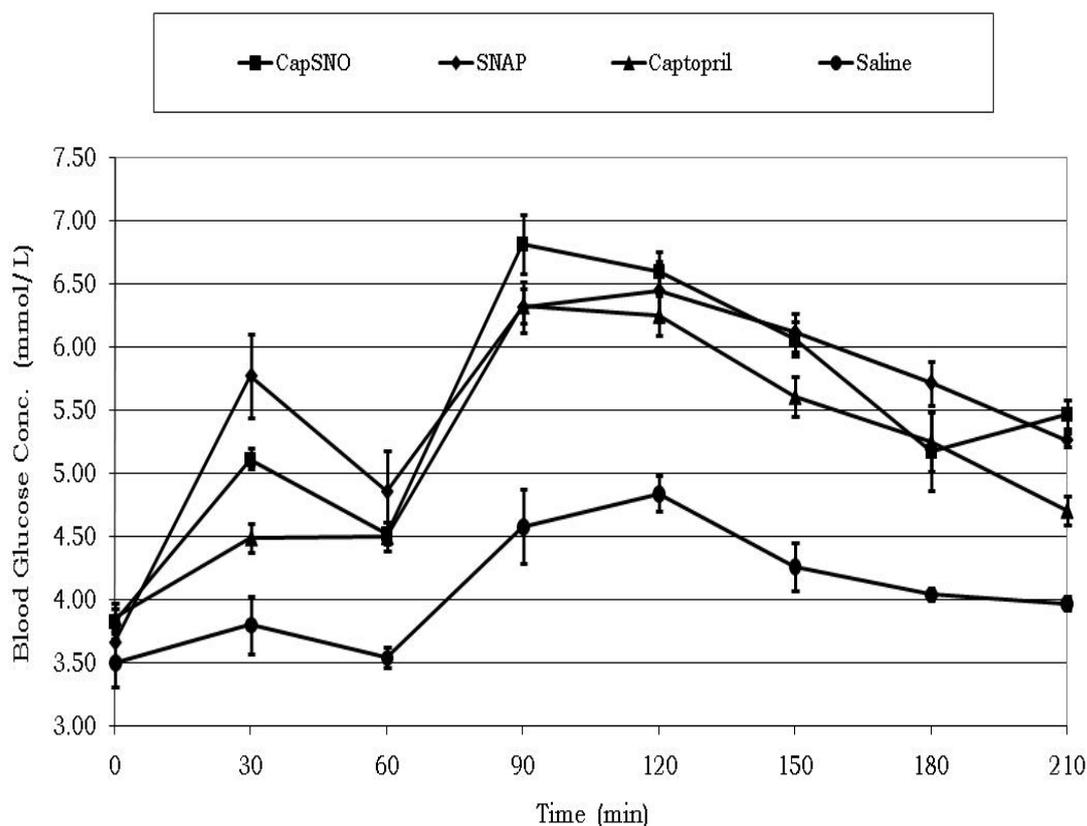


Fig. 1. Effect of CapSNO, SNAP and captopril on fasting and postprandial blood glucose concentration.

captopril were better able to attain normal glucose tolerance when compared with rats treated with the same dosage of CapSNO and SNAP.

The effect of CapSNO and SNAP vs captopril on haemodynamic parameters

The effect of CapSNO, SNAP and captopril on haemodynamic parameters were also investigated. Baseline systolic/diastolic blood pressure readings of 128/96 mmHg, 115/79 mmHg, 120/73 mmHg were obtained for SNAP, CapSNO and captopril-treated rats respectively. Saline treated rats (control) had a baseline reading of 108/91. In captopril-treated rats there was a significant decrease in systolic blood pressure (SBP) from 119.63 ± 3.75 mmHg at the -10 min interval to 102.88 ± 4.85 mmHg at the 5 min interval (significant Table 1). This decrease in SBP was maintained for 15 min. Rats that were administered CapSNO and SNAP also showed a significant decrease in SBP after the compound was administered, however the effect was maintained for a much longer period. The data showed that

SNAP-treated rats gave a significant decrease in SBP from 128.10 ± 3.96 mmHg (-10 min) to 110.57 ± 4.54 mmHg (5 min significant). This reduction lasted until the 20 min interval where there was a return to a near baseline value of 127.20 ± 3.47 mmHg. CapSNO treated rats showed a more significant decrease after the compound was administered when compared to rats treated with SNAP only. The data showed that the SBP decreased from 114.75 ± 2.66 mmHg at the -10 min interval to 90.53 ± 2.59 mmHg (5 min significant). The reduction in blood pressure also lasted until the 20 min interval.

A significant decrease in diastolic blood pressure (DBP) was also observed in rats treated with CapSNO, SNAP and captopril (Table 2). In SNAP and captopril-treated rats, there was a decrease in DBP from 94.70 ± 2.83 mmHg and 76.37 ± 1.06 mmHg (-10 min) to a minimum of 80.40 ± 2.55 mmHg and 62.27 ± 3.77 mmHg respectively, 10 min after the compounds were administered. However at the 20 min interval, the DBP returned to near baseline i.e., 92.00 ± 2.42 mmHg for SNAP-treated rats. For

captopril-treated rats the DBP returned to a near baseline value of 76.81 ± 3.01 mmHg, 15 min after the compound was administered. Rats that were treated with CapSNO showed the most significant decrease in DBP as there was a decrease from 77.10 ± 3.19 mmHg (-10 min) to 64.42 ± 1.04 mmHg, 5 minutes after the compound was administered (significant). In addition, the reduction in DBP was maintained throughout the experiment. CapSNO was therefore shown to be the most potent of the three compounds in causing a reduction in the DBP.

The significant decrease in SBP and DBP obtained in rats treated with CapSNO, SNAP and captopril was accompanied by an increase in heart rate (Table 3). The typical heart rate for Wistar and Sprague-Dawley pure strain rats is 330–360 beats per minute (BPM). The data obtained for captopril-treated rats showed a significant increase in heart rate from 382.23 ± 21.83 BPM (-10 min) to a maximum of 825.89 ± 72.54 BPM at the 10 min interval (significant). There was a gradual decrease thereafter to 391.71 ± 7.92 BPM at the 55 min interval. For CapSNO-treated rats the heart rate showed a significant increase from 379.25 ± 19.54 BPM (-10 min) to a value of 544.42 ± 43.43 BPM at the 30 min interval. This increase was maintained for the duration of the experiment. SNAP-treated rats showed an increase from 365.10 ± 6.44 BPM (-10 min) to 565.28 ± 3.81 BPM at the 5 min interval, however as time progressed the heart rate was significantly reduced and near normal heart rate was attained 35 min after the compound was administered. The elevated heart rate was more pronounced in captopril-treated rats compared with SNAP and CapSNO-treated rats. In addition this elevation in heart rate was more significant in CapSNO-treated rats when compared to SNAP-treated rats.

DISCUSSION

S-nitrosothiols are formed both *in vivo* and *in vitro* from their corresponding thiols (Van der Vliet et al. 1998). The effect of CapSNO, SNAP and captopril on fasting and postprandial blood glucose concentrations was investigated to determine whether nitrosylation of a thiol affected normal glucose tolerance. The data showed that both CapSNO and SNAP at 12.5 mg/kg BW caused a significant elevation of fasting blood glucose concentration compared to captopril at the same dosage. Both CapSNO and SNAP caused a dose-dependent elevation of blood glucose concentration. The effect of RSNOs on blood glucose concentration correlates with findings by McGrowder

et al. (1999, 2001) who showed that S-nitrosoglutathione (GSNO) and SNAP caused a dose dependent increase in plasma glucose levels in normoglycaemic dogs. The effect was accompanied by a decrease in plasma insulin levels.

Some researchers have proposed that captopril does not have a significant effect on blood glucose concentration (Winocour et al. 1986, McGrowder et al. 2003). However the data obtained showed that captopril is able to cause a dose-dependent increase in the blood glucose concentration. The postprandial data showed a steady increase in the blood glucose concentration when dosages of 7.0, 12.5 and 20.0 mg/kg BW were administered. It is possible that dosages higher than 20 mg/kg BW of captopril could result in impaired glucose tolerance.

The effects of CapSNO and SNAP on blood glucose concentration were compared with captopril. Both CapSNO and SNAP showed a significant increase in the blood glucose concentration when compared with captopril-treated rats. Captopril is unable to permeate the cell membrane (Jia and Wong 2001) and does not decompose and liberate NO. This explains the significant difference observed in rats that were treated with captopril compared with CapSNO and SNAP-treated rats. The main difference in the structure between the captopril and the RSNOs is the presence of a thionitrite functional group which is found only in the latter group. CapSNO is derived from captopril and still retains its ACE inhibitor properties (Park and Suzuki 2007), however it affects blood glucose concentration in a different manner. The presence of the thionitrite group is responsible for the hyperglycaemic effect observed and, generally, the activity of the CapSNO (Matthew and Kerr 1993).

S-nitrosocaptopril and SNAP belong to different class structures having a primary and a tertiary structure respectively. Due to the bulky structure of SNAP, it would more readily decompose (Aqart and Dasgupta 2004) via transnitrosation, liberating NO (Myers et al. 1990, Liu et al. 1998). This reaction is facilitated by the presence of reductants (Singh et al. 1996b) that are found in trace quantities *in vivo*. S-nitrosocaptopril is able to permeate cell membrane (Jia and Wong 2001), however it is not clear as to whether SNAP is also able to permeate these barriers. There exists a possibility that the intact CapSNO molecule may be responsible for the effect observed as some studies have shown that RSNOs in their intact form and the decomposed product, are both able to activate soluble guanylate cyclase (sGC) (Craven and DeRubertis 1983). In addition there exists no correlation between the stability of RSNOs and their activity (Matthew and Kerr 1993). There was a significant increase in fasting blood glucose

Table 1. Effect of CapSNO, SNAP and captopril on systolic blood pressure.

Time (min)	Systolic blood pressure (mmHg)			
	CapSNO	SNAP	Captopril	Saline
-5	106.61 ± 3.12	140.70 ± 5.30	115.70 ± 3.19	102.75 ± 6.26
-10	114.75 ± 2.66	128.10 ± 3.96	119.63 ± 3.75	107.50 ± 5.20
5	90.53 ± 2.59*	110.57 ± 4.54*	102.88 ± 4.85	113.00 ± 3.14
10	95.45 ± 0.99	120.70 ± 4.00	105.89 ± 4.13	120.25 ± 2.30
15	105.70 ± 2.00	117.80 ± 2.00	120.33 ± 4.39*	114.00 ± 3.76
20	113.42 ± 2.94	127.20 ± 3.47*	124.50 ± 4.74	120.25 ± 2.46
25	109.28 ± 2.35	131.10 ± 3.02	122.37 ± 2.17	108.75 ± 1.25
30	110.42 ± 3.07	132.80 ± 4.27	126.38 ± 3.20	115.00 ± 1.13
35	107.40 ± 0.89	137.00 ± 5.76	125.76 ± 2.37	129.78 ± 3.49
40	117.27 ± 2.47	128.80 ± 2.27	118.00 ± 3.31	122.76 ± 2.58
45	113.33 ± 1.33	140.70 ± 1.87	118.97 ± 4.15	122.89 ± 2.58
50	107.76 ± 3.23	135.80 ± 4.59	121.14 ± 2.22	115.29 ± 3.27
55	105.35 ± 1.85	123.60 ± 0.70	122.50 ± 1.79	126.17 ± 3.22

* Current time statistically significant vs previous

Table 2. Effect of CapSNO, SNAP and captopril on diastolic blood pressure.

Time (min)	Diastolic blood pressure (mmHg)			
	CapSNO	SNAP	Captopril	Saline
-5	78.93 ± 3.13	95.82 ± 4.01	72.63 ± 1.27	90.75 ± 6.20
-10	77.10 ± 3.19	94.70 ± 2.83	76.37 ± 1.06	97.25 ± 3.24
5	64.42 ± 1.04*	86.29 ± 2.97*	65.56 ± 2.76*	97.00 ± 2.56
10	70.10 ± 1.29	80.40 ± 2.55	62.27 ± 3.77	94.50 ± 1.50
15	74.90 ± 1.22	83.40 ± 2.50	76.81 ± 6.01*	89.75 ± 2.32
20	72.42 ± 3.24	92.00 ± 2.42*	70.50 ± 1.67	86.00 ± 1.54
25	70.06 ± 2.63	94.20 ± 1.33	78.15 ± 1.67	84.50 ± 1.36
30	64.05 ± 2.82	94.00 ± 2.45	80.89 ± 1.35	83.20 ± 1.52
35	70.30 ± 1.68	96.75 ± 5.19	79.86 ± 1.23	91.91 ± 3.39
40	70.93 ± 3.30	96.60 ± 1.89	75.60 ± 2.06	94.60 ± 5.38
45	68.83 ± 2.16	102.00 ± 2.06	74.90 ± 1.71	87.60 ± 2.33
50	63.71 ± 2.73	101.30 ± 2.79	78.96 ± 1.99	91.83 ± 2.74
55	62.80 ± 2.06	94.50 ± 0.82	82.32 ± 1.55	86.00 ± 3.13

Symbols as in Table 1

Table 3. Effect of CapSNO, SNAP and captopril on heart rate.

Time (min)	Heart Rate (beats per minute)			
	CapSNO	SNAP	Captopril	Saline
-5	602.08 ± 56.76	406.10 ± 22.23	510.81 ± 55.49	337.25 ± 10.71
-10	379.25 ± 19.54	365.10 ± 6.44	382.23 ± 21.83	327.00 ± 6.62
5	472.95 ± 6.19*	565.28 ± 3.81*	686.12 ± 98.13*	351.75 ± 13.31
10	403.00 ± 8.54	451.10 ± 7.61	825.89 ± 72.54	352.25 ± 15.12
15	346.85 ± 6.77	405.30 ± 6.34	669.33 ± 69.77	338.75 ± 29.38
20	351.00 ± 34.93	413.60 ± 25.31	595.32 ± 42.03	344.50 ± 21.25
25	370.83 ± 29.58	411.30 ± 29.18	569.44 ± 33.50	339.75 ± 19.40
30	544.42 ± 43.43	404.60 ± 15.07	498.83 ± 38.82	330.00 ± 17.91
35	388.45 ± 49.28	380.00 ± 7.88	426.66 ± 14.88	379.39 ± 23.61
40	552.33 ± 75.95	350.80 ± 17.42*	416.65 ± 15.02	312.45 ± 52.68
45	393.28 ± 31.04	380.20 ± 10.24	400.73 ± 8.61	316.33 ± 32.21
50	544.18 ± 80.35	350.80 ± 2.79	403.56 ± 9.22	316.33 ± 55.35
55	461.85 ± 35.87	349.70 ± 3.68	391.71 ± 7.92	364.17 ± 35.09

Symbols as in Table 1

concentration observed in SNAP-treated rats, after SNAP was administered. This suggests that SNAP was faster acting and as such was able to elicit the hyperglycaemic response once in the blood stream, possibly by a mechanism that involves the release of NO. The inability of CapSNO to also elicit a fast acting response may be linked to the stability of this compound *in vivo* (Nakae et al. 1995). The postprandial data however showed no significant difference in the effect of both CapSNO and SNAP.

Many people who suffer from chronic diabetes are also affected by hypertension. Therefore there exists the need for drugs that are able to treat both conditions. S-nitrosothiols have been considered as a replacement for organic nitrates and inorganic nitrovasodilators as the search for new 'NO donor drugs' continues, because of the numerous disadvantages associated with these vasodilators when compared with RSNOs. The development of nitrate tolerance is one of the major limitations associated with the use of organic nitrates. The mechanism involves the oxidation of critical sulfhydryl groups in the vascular smooth muscle receptors forming a dimer which will have a lower affinity for the drug (Needleman and Johnson 1973, Feelisch and Kelm 1991). Tolerance will develop in the smooth muscle unless adequate stores of reduced intracellular thiol are maintained (Loscalzo et al. 1989). However reports have shown that thiol supplementation did not alter the vasorelaxation caused by nitrates (Henry et

al. 1989a, b). In addition this group of compound releases NO by a complex mechanism thus reducing its potency. The release of NO from organic nitrates only occurs after the compound has been bio-transformed. The mechanism involves the reduction of the nitrate portion of the compound to nitrite by tissue thiols, followed by nitrosation to form RSNO, which decomposes, liberating NO (Henry et al. 1989c). The formation of toxic metabolites is a major risk factor associated with the use of inorganic nitrovasodilators. Physiological degradation of sodium nitroprusside (SNP) results in an increased level of cyanide in the blood. As a result the duration of intravenous infusion of SNP is limited to less than 72 h (Megson 2000). The reaction of the nitroprusside with thiols is thought to be the mechanism by which cyanide is released from this compound. Studies investigating two possible non-enzymatic chemical reactions i.e. the reaction with free thiols and with haemoglobin showed that the latter was significantly responsible for cyanide release (Smith and Kruszyna 1974).

RSNOs are potent vasodilators and are able to cause a significant decrease in diastolic blood pressure and systolic blood pressure (Cooke et al. 1989, Henry et al. 1989b). The mechanism involves the release of the NO from the RSNOs leading to the activation of sGC and a decrease in Ca²⁺ concentration (Ignarro and Kadowitz 1985, Ignarro et al. 1999). Some researchers have shown that the ability to cause smooth muscle relaxation may not be due to the release of NO from

the RSNO (Myers et al. 1990). The initiation of this mechanism allows the blood to flow smoothly through the blood vessels. Captopril is a potent ACE inhibitor and is ideal for use in treating hypertension but the duration of the vasodilatory effect was short in comparison to the effect caused by the RSNOs. Other studies have shown that CapSNO and SNAP are more potent than captopril (Shaffer et al. 1991, Nakae et al. 1995) in causing a reduction in blood pressure. Captopril was shown to be less efficient in reducing hypertension even at a 10-fold higher concentration than CapSNO (Shaffer et al. 1991). According to Nakae et al. (1995), the effect of CapSNO as a vasodilatory agent more closely resembles organic nitrate nitroglycerine (NTG) than its thiol counterpart, therefore the ACE activity does not play a significant part in this role.

Analysis of the haemodynamic data revealed that CapSNO and SNAP were more effective in causing a reduction in blood pressure when compared with captopril. According to the data obtained, CapSNO caused the most significant decrease in blood pressure and was shown to be more potent than SNAP and captopril. The decrease in blood pressure may therefore be due to a synergistic effect. The data correlates with findings proposed by Shaffer et al. (1991) who showed that CapSNO administered at a dosage of 12.5 mg/kg BW was able to significantly decrease blood pressure in anaesthetized and conscious rats when compared with captopril-treated rats. The decrease in blood pressure was accompanied by tachycardia. This elevation in heart rate was most significant in captopril-treated rats (Nakae et al. 1995). CapSNO is able to act both as an ACE inhibitor and a nitrovasodilator (Loscalzo et al. 1989, Park and Suzuki 2007) thus explaining why this molecule was shown to be more potent than SNAP and captopril in causing a reduction in blood pressure.

The ability of RSNOs such as CapSNO and SNAP to significantly increase blood glucose concentration may limit their use as anti-hypertensive agents for individuals unable to regulate normal glucose tolerance. However if the RSNO is co-administered with an antihyperglycaemic agent, then its use may be beneficial to such individuals.

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