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Stereoselective *S*-nitrosocysteine recognition sites in rat brain

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Abstract

We examined the effects of intracisternal (i.c.) injections (10–250 nmol) of the L- and D-isomers of *S*-nitrosocysteine (L- and D-*S*-nitrosocysteine) on the mean arterial blood pressure and heart rate of conscious rats, and the decomposition of L- and D-*S*-nitrosocysteine to nitric oxide (NO) upon addition to brain homogenates. The i.c. injection of L-*S*-nitrosocysteine produced initial falls in mean arterial blood pressure and heart rate which were followed by increases in these parameters. The i.c. injection of D-*S*-nitrosocysteine did not produce initial falls in mean arterial blood pressure or heart rate but produced the subsequent increases in these parameters. L- and D-*S*-nitrosocysteine decomposed equally to NO. These results suggest that the initial effects of L-*S*-nitrosocysteine may be due to the activation of stereoselective recognition sites on brain neurons.

Keywords: *S*-Nitrosocysteine; Stereoisomer; Nitric oxide (NO)

Microinjections of the nitric oxide (NO)-containing factor *S*-nitrosocysteine into the nucleus tractus solitarius produce hypotension in anesthetized (Lewis et al., 1991) and conscious (Machado and Bonagamba, 1992) rats. The possibility that *S*-nitrosocysteine may exert its effects via the activation of membrane-bound receptors which specifically recognize this *S*-nitrosothiol has not been addressed. The stereoisomeric configuration of an agonist usually determines binding or agonist potency at the receptor (Stitzel and Robinson, 1990). As such, differences in the potencies of the L- and D-isomers of *S*-nitrosocysteine (L- and D-*S*-nitrosocysteine) would suggest the presence of stereoselective *S*-nitrosocysteine recognition sites, especially if it could be established that the stereoisomers decompose equally to NO. We examined the changes in mean arterial blood pressure and heart rate of conscious rats produced by the intracisternal (i.c.) injection of 10–250 nmol of (1) L- and D-*S*-nitrosocysteine, (2) L-cystine, (3) L- and D-cysteine, and (4) L-*S*-nitroso-γ-glutamylcysteinylglycine (L-*S*-nitrosoglutathione). We also examined the decompostion of L- and D-*S*-nitrosocysteine and L-*S*-nitrosoglutathione to NO upon their addition to brain homogenates in artificial cerebrospinal fluid (artificial CSF).

These studies were approved by the University of Iowa Animal Care and Use Committee. Male Sprague-Dawley rats (300–350 g) were anesthetized with acepromazine maleate (12 mg/kg i.p.) and ketamine (120 mg/kg i.p.) and implanted with an injection assembly (20 gauge) into the cisterna magna. Catheters (PE-50) were implanted into the left femoral artery for the measurement of mean arterial blood pressure and heart rate. After a 7-day recovery period, the animals were connected to a Beckman Dynograph for the recording of mean arterial blood pressure and heart rate. After allowing the rats several minutes to stabilize, they received i.c. injections of 0.5–1 μl of the test compounds (10–250 nmol). The effects of L- and D-*S*-nitrosocysteine and vehicle (methanol) were examined in 8 rats. The effects of L-cystine and L-*S*-nitrosoglutathione ($n = 5$) and L- and D-cysteine ($n = 5$) were examined in other groups of rats. Another 12 rats were anesthetized and perfused transcardially with ice-cold 0.1 M phosphate-buffered saline (pH 7.4) (PBS) and the brains removed and placed in 5 ml of PBS and then homogenized, centrifuged and the pellet collected. 10 μl of methanol containing 50–1250 nmol of L- or D-*S*-nitrosocysteine or L-*S*-nitrosoglutathione were injected into sealed 12-well plates containing 90 μl of artificial CSF

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plus 100 mg of the brain homogenate. The NO released from the *S*-nitrosothiols was carried to the NO detector by a stream of N_2 gas (Myers et al., 1990). The initial concentrations of L- and D-*S*-nitrosocysteine and L-*S*-nitrosoglutathione in the wells were 1–12.5 mM. These concentrations were chosen because the injection of 10–250 nmol of the *S*-nitrosothiols would reach peak concentrations of 0.5–12.5 mM in the CSF which has a volume of approximately 20 μl. L- and D-*S*-nitrosocysteine and L-*S*-nitrosoglutathione were prepared by the reaction of 2 mM L- or D-cysteine or L-glutathione with 2 mM nitrogen dioxide gas in 1 ml of cold methanol (Myers et al., 1990). The data are presented as mean ± S.E.M. and were analyzed by repeated-measures analysis of variance followed by Student's modified *t*-test with the Bonferroni correction for multiple comparisons between means.

The resting mean arterial blood pressure values for the three groups of rats were 116 ± 4, 118 ± 3 and 123 ± 5 mm Hg ($P > 0.05$ for all comparisons). The resting heart rate values for these groups were 326 ± 15, 348 ± 16 and 339 ± 12 ($P > 0.05$ for all comparisons). The initial effects on mean arterial blood pressure and heart rate produced by the i.c. injections of L- and D-*S*-nitrosocysteine are summarized in Fig. 1. L-*S*-Nitrosocysteine produced dose-dependent reductions in these parameters which lasted for 30–60 s. D-*S*-Nitrosocysteine did not produce these initial falls in mean arterial blood pressure or heart rate. The initial L-*S*-nitrosocysteine-induced falls in mean arterial blood pressure and heart rate were followed by increases in these parameters which were sustained for 1–3 min. The increases in mean arterial blood pressure produced by the 10, 50, 100 and 250 nmol doses of L-*S*-nitrosocysteine were +1 ± 1, +4 ± 2, +10 ± 3 and +16 ± 3 mm Hg, respectively ($P < 0.05$, for higher two doses) and the increases in heart rate were +3 ± 2, +7 ± 4, +18 ± 4 and +27 ± 5 beats/min, respectively ($P < 0.05$, for higher two doses). D-*S*-Nitrosocysteine produced similar delayed increases in mean arterial blood pressure and heart rate. The increases in mean arterial blood pressure produced by the 10–250 nmol doses of D-*S*-nitrosocysteine were +2 ± 1, +3 ± 2, +13 ± 4 and +18 ± 4 mm Hg, respectively ($P < 0.05$, for higher two doses) and the increases in heart rate were +6 ± 4, +10 ± 6, +22 ± 5 and +30 ± 7 beats/min, respectively ($P < 0.05$, for higher two doses). The i.c. injection of methanol (0.5–1.0 μl, $n = 8$) did not alter mean arterial blood pressure or heart rate ($P > 0.05$ for all comparisons). The i.c. injection of L-*S*-nitrosoglutathione did not produce the initial falls in mean arterial blood pressure or heart rate ($P > 0.05$ for all comparisons) but did produce the delayed increases in these parameters. The increases in mean arterial blood pressure produced by the 10–250 nmol doses of L-*S*-nitrosoglutathione were +1 ± 2, +2 ± 2, +11 ± 4 and +15 ± 4 mm Hg, respectively ($P < 0.05$, for higher two doses) and the increases in heart rate were +2 ± 2, +13 ± 6, +16 ± 3 and +23 ± 5 beats/min, respectively ($P < 0.05$, for higher two doses). The i.c. injection of L-cystine or L- or D-cysteine did not affect mean arterial blood pressure or heart rate ($P > 0.05$ for all comparisons). The total NO detected upon the application of L- or D-*S*-nitrosocysteine or L-*S*-nitrosoglutathione to brain homogenates in artificial CSF are summarized in the bottom panel of Fig. 1. The addition of the *S*-nitrosothiols to the brain homogenates resulted in the concentration-dependent appearance of NO which was detected within 1–2 s. Each concentration of L- and D-*S*-nitrosocysteine and L-*S*-nitrosoglutathione generated similar amounts of NO.

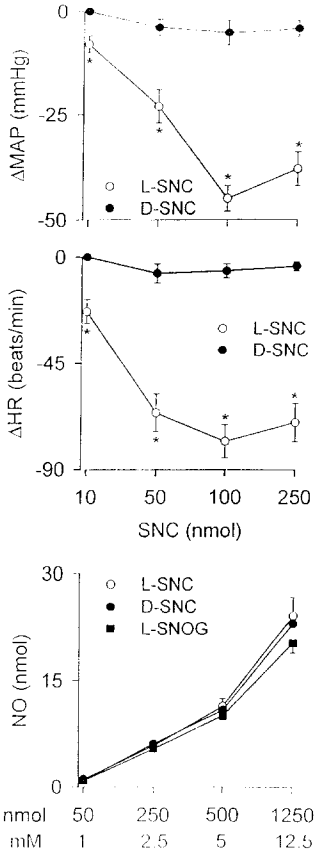

Fig. 1. Top two panels: A summary of the effects of i.c. injections of L- or D-*S*-nitrosocysteine (L- and D-SNC, 10–250 nmol) on the mean arterial blood pressures (MAP) and heart rates (HR) of conscious freely moving rats ($n = 8$). Each value represents the mean ± S.E.M. of the arithmetic changes in these parameters. * $P < 0.05$, significant response. Bottom panel: The total amount of NO detected after the addition of L- or D-*S*-nitrosocysteine (L- or D-SNC) or L-*S*-nitrosoglutathione (L-SNOG) to brain homogenates. Each value represents the mean ± S.E.M. of the arithmetic changes in these parameters. The NO was recorded from three individual experiments (3 replicates per experiment).

This study demonstrates that the i.c. injection of L-*S*-nitrosocysteine produced initial falls in mean arterial blood pressure and heart rate in conscious rats whereas D-*S*-nitrosocysteine or L-*S*-nitrosoglutathione did not produce these responses. We also found that L- and D-*S*-nitrosocysteine and L-*S*-nitrosoglutathione decomposed equally to NO upon addition to brain homogenates. These results suggest that the initial falls in mean arterial blood pressure

and heart rate produced by L-*S*-nitrosocysteine depends upon its stereoisomeric configuration rather than to its decomposition to NO. The finding that L-*S*-nitrosoglutathione did not produce initial falls in mean arterial blood pressure and heart rate suggests that the putative *S*-nitrosocysteine receptor sites are not activated by *S*-nitrosothiols of substantially different structure to L-*S*-nitrosocysteine. The findings that L-cystine and L- and D-cysteine did not affect mean arterial blood pressure or heart rate further suggests that the L-*S*-nitrosocysteine moiety is responsible for the initial falls in mean arterial blood pressure and heart rate. L- and D-*S*-nitrosocysteine and L-*S*-nitrosoglutathione produced similar delayed increases in mean arterial blood pressure and heart rate. These responses may be due to the breakdown of these *S*-nitrosothiols to NO. In addition, *S*-nitrosothiols modulate neuronal function by the transnitrosation of cysteine residues of the redox site of the *N*-methyl-D-aspartate (NMDA) receptor (Lei et al., 1992). Therefore, it is possible that the delayed increases in mean arterial blood pressure and heart rate produced by the *S*-nitrosothiols may involve transnitrosation processes.

Taken together, our findings raise the possibility that L-*S*-nitrosocysteine may alter neuronal function by the activation of stereoselective recognition sites in the membranes of these cells. These recognition sites may represent membrane-bound receptors for which *S*-nitrosocysteine is the preferred agonist. This possibility is supported by a preliminary receptor-binding study which demonstrated binding sites for L-*S*-nitrosoglutathione in the brain (Taguchi et al., 1995). The presence of these putative *S*-nitrosocysteine receptors suggests that NO synthase-containing neurons (Dawson et al., 1992) may synthesize *S*-nitrosocysteine or closely related *S*-nitrosothiols. Although there is no evidence that *S*-nitrosocysteine is synthesized by neurons in the CNS, a recent preliminary report provided evidence that L-*S*-nitrsosoglutathione exists in the rat cerebellum (Kluge et al., 1995).

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