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Effect of intracerebroventricular and intravenous administration of nitric oxide donors on blood pressure and heart rate in anaesthetized rats

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1 The effects of nitric oxide (NO) releasing substances, sodium nitroprusside, 3-morpholino sydnonimine (SIN-1) and a novel oxatriazole derivative, GEA 3162, on blood pressure and heart rate were studied after peripheral or central administration in anaesthetized normotensive Wistar rats.

2 Given as cumulative intravenous injections, both nitroprusside and GEA 3162 (24–188 nmol kg^{-1}) induced short-lasting and dose-dependent decreases in mean arterial pressure, while SIN-1 decreased blood pressure only slightly even after larger doses (94–3000 nmol kg^{-1}). Heart rate increased concomitantly with the hypotensive effect of the NO-releasing substances.

3 Cumulative intracerebroventricular administration of GEA 3162 (24–188 nmol kg^{-1}) induced a dose-dependent hypotension with slight but insignificant increases in heart rate. In contrast, intracerebroventricular nitroprusside induced little change in blood pressure, while a large dose of SIN-1 (3000 nmol kg^{-1}, i.c.v.) slightly increased mean arterial pressure. However, intracerebroventricular nitroprusside and SIN-1 increased heart rate at doses that did not significantly affect blood pressure.

4 To determine whether the cardiovascular effects of GEA 3162 were attributable to an elevation of cyclic GMP levels, pretreatments with methylene blue, a putative guanylate cyclase inhibitor, were performed. This substance failed to attenuate the cardiovascular effects of peripherally or centrally administered GEA 3162, suggesting that the effects were independent of guanylate cyclase.

5 In conclusion, the centrally administered NO-donor, GEA 3162, induced a dose-dependent hypotensive response without significant changes in heart rate. Furthermore, intracerebroventricular injections of nitroprusside and SIN-1 increased heart rate without affecting blood pressure. These results suggest that NO released by these drugs may affect central mechanisms involved in cardiovascular regulation independently of cyclic GMP.

Keywords: NO-donors; nitroprusside; SIN-1; GEA 3162; methylene blue; LY-83583; central nervous system

Introduction

Nitric oxide (NO) is involved in the control of peripheral vascular tone, platelet aggregation, and macrophage-induced cytotoxicity via stimulation of the soluble guanylate cyclase (for review, see Moncada *et al.*, 1991). Furthermore, NO is most likely the final effector molecule of nitrovasodilators that activate the soluble guanylate cyclase, e.g. organic nitrates, sodium nitroprusside, and sydnonimines such as SIN-1, the active metabolite of the anti-anginal prodrug, molsidomine.

NO is synthesized from L-arginine in peripheral tissues and cells in a reaction catalysed by isoenzymes of NO synthase (Moncada *et al.*, 1991). NO synthase is also widely distributed in specific neuronal populations in the brain (Bredt *et al.*, 1990; Vincent & Kimura, 1992). However, the role of NO in the central nervous system (CNS) is at present largely unknown. NO production in the brain has been linked to excitatory amino acid receptor activation. Stimulation of N-methyl-D-aspartate (NMDA) receptors by glutamate was shown to evoke NO formation which in turn activated guanylate cyclase and thereby increased guanosine 3',5'cyclic-monophosphate (cyclic GMP) levels in the cerebellum (Garthwaite, 1991). It has been proposed that NO acts as an intercellular messenger which contributes to the induction of synaptic plasticity (Shibuki & Okada, 1991). There is also experimental evidence that NO modulates e.g. drinking behaviour (Calapai *et al.*, 1992), nociception (Moore *et al.*, 1991), integrative respiratory function (Ling *et al.*, 1992), and wakefulness (Kapas *et al.*, 1994). Moreover, NO is regarded as a neurotransmitter in autonomic non-adrenergic non-cholinergic (NANC) nerves (Bredt *et al.*, 1990). Thus, NO seems to be a neural messenger in central and peripheral nervous systems.

The L-arginine-NO pathway in the brain may also affect central mechanisms involved in cardiovascular regulation. Inhibition of endogenous NO formation in various brain areas has been shown to increase blood pressure and sympathetic nerve activity in different species, including cats, rabbits and rats (Shapoval *et al.*, 1991; Togashi *et al.*, 1992; Cabrera & Bohr, 1995). However, there are some differences in the results after central administration of NO-releasing substances. While administration of glyceryl trinitrate, nitroprusside or DEA/NO into the lateral cerebral ventricle decreased blood pressure in anaesthetized cats and rats (Ma & Long, 1992; Hedge *et al.*, 1994; Cabrera & Bohr, 1995), another NO-donor, S-nitroso-N-penicillamine (SNAP) has been reported to produce a slight increase in blood pressure in conscious rats (Ota *et al.*, 1993).

In order to study further the possible central cardiovascular effects of NO, we evaluated the effects of various NO-releasing substances on blood pressure and heart rate after intracerebroventricular administration in anaesthetized rats. For comparison of central and peripheral effects, intravenous administration was also carried out. Earlier known nitrovasodilators which yield NO, sodium nitroprusside (Bates *et al.*, 1991) and SIN-1 (Feelisch *et al.*, 1989), as well as a new potent NO-donor, a mesoionic oxatriazole derivative, GEA 3162, were used in the experiments. This new GEA compound has been demonstrated to release NO in aqueous solutions by several techniques (Karup *et al.*, 1994; Kankaanranta *et al.*, 1996).

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Methods

Experimental procedures

The studies were carried out on normotensive male Wistar rats (230–290 g). The experimental protocol was approved by the Animal Experimentation Committee of the Institute of Biomedicine, University of Helsinki. Rats were anaesthetized with urethane (1.5 g kg^{-1}, i.p.) and the trachea was cannulated with a polyethylene tube in order to facilitate spontaneous respiration. Rectal temperature was kept constant at 37±0.3°C by a heating pad placed under the animal. The left femoral artery was cannulated for monitoring of mean arterial pressure (MAP). The arterial cannula containing heparinized saline was connected to a pressure transducer (Buxco Electronics Inc., Model CVA 1, U.S.A.) calibrated with a recording laboratory computer. Heart rate (HR) was obtained from the blood pressure pulses. The left femoral vein was cannulated for intravenous (i.v.) injections.

The rats were placed in a stereotaxic device for the cannulation of the lateral cerebral ventricle. A 26 G steel cannula connected to a Hamilton syringe was used for intracerebroventricular (i.c.v.) administration of drugs. Coordinates for the injections were 5.3 mm posterior and 4.0 mm lateral relative to the bregma and 2.0 mm vertical from the dura. After the placement of the cannula at least 30 min were allowed for stabilization before beginning the experiment. Correct placement of the intracerebroventricular cannula was verified by staining with methylene blue after completion of the experiments.

Cumulative doses of NO-donors or saline were administered intravenously or intracerebroventricularly at 10–15 min intervals in a volume of 0.1 ml and 10 μl, respectively. Control animals received an equivalent volume of saline (0.9% NaCl) repeatedly over the same period of time. In separate series of experiments, peripheral and central pretreatments with methylene blue and LY-83583 were performed 3–10 min prior to the administration of GEA 3162.

Drugs

GEA 3162 (3-aryl-1,2,3,4-oxatriazole-5-imine) and SIN-1 (3-morpholino sydnonimine) were generously donated by GEA Ltd. (Copenhagen, Denmark). Sodium nitroprusside (Roche, Basel, Switzerland) was purchased from the University Pharmacy (Helsinki, Finland). Methylene blue was obtained from BDH Ltd. (Poole, U.K.) and LY-83583 (6-anilinoquinoline-5,8-quinone) from Calbiochem-Novobiochem Corp. (La Jolla, CA, U.S.A.).

Solutions of NO-releasing substances were prepared immediately before use. GEA 3162 and SIN-1 were dissolved in saline (0.9% NaCl). Sodium nitroprusside was dissolved in 5% glucose solution and thereafter diluted in saline.

Statistics

All values are presented as mean±s.e.mean. The changes in MAP and HR are relative to the initial baseline level within 5 min before the administration of the drugs. The results were analysed by repeated measures analysis of variance (ANOVA) corrected for multisample asphericity by the Huynh-Feldt correction. Comparisons to the initial baseline levels were performed by Student's paired t test adjusted by the Bonferroni correction for multiple comparisons (Ludbrook, 1994). The effects of GEA 3162 on MAP or HR after pretreatment with methylene blue or LY-83583 were compared to corresponding vehicle-pretreated control groups by Student's unpaired t test. P values <0.05 were considered statistically significant. SYSTAT for Windows (Systat Inc., Evanston, IL, U.S.A.) was used for statistical calculations.

Results

There were no statistically significant differences with respect to the initial baseline values for MAP and HR among the various experimental groups.

Cardiovascular effects of NO-releasing substances after intravenous administration

Nitroprusside and GEA 3162 (24–188 nmol kg^{-1}, i.v.) dose-dependently reduced MAP after cumulative intravenous administrations ($P<0.001$, repeated measures ANOVA within the groups) (Figure 1). The decrease in blood pressure peaked within 30–45 s and lasted less than 2 min after all doses. With the largest dose of 188 nmol kg^{-1}, i.v., MAP decreased maximally to 44±2 mmHg from an initial baseline value of 79±4 mmHg after nitroprusside, whereas following the same dose of GEA 3162 the maximal fall in MAP was to 44±3 mmHg from an initial value of 76±4 mmHg. Both substances also increased HR ($P<0.05$ for nitroprusside and $P<0.01$ for GEA 3162). The increases in HR peaked within 2 min and returned to the preadministration level during the 10 min interval between the cumulative injections, except after the largest dose of 188 nmol kg^{-1}, i.v. (Figure 1). No significant differences in MAP or HR responses between the two drugs were observed. There was no obvious development of tolerance to the depressor effects after repeated applications of nitroprusside or GEA 3162.

Cumulative intravenous doses of SIN-1 (94–3000 nmol kg^{-1}) moderately decreased blood pressure ($P<0.05$) with concomitant increases in HR ($P<0.05$) (Figure 2). With lower doses of SIN-1 (24 and 47 nmol kg^{-1}, i.v.) no effects on MAP or HR were obtained (data not shown). At the largest dose of 3000 nmol kg^{-1}, i.v., MAP reduced to 58±2 mmHg from an initial baseline value of 78±2 mmHg, and HR increased to 443±26 beats min^{-1} from a mean base-

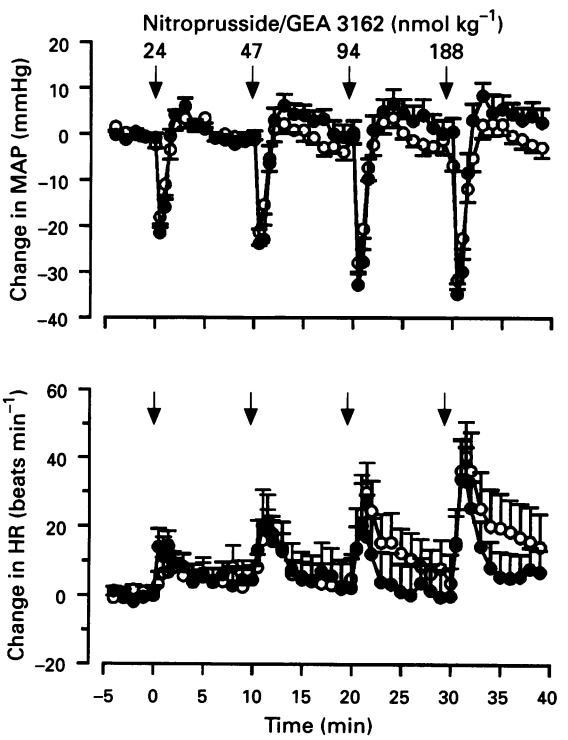

Figure 1 Time-course showing the effect of cumulative intravenous doses (24–188 nmol kg^{-1}) of sodium nitroprusside (●) and GEA 3162 (○) on mean arterial pressure (MAP) and heart rate (HR). Initial baseline values for MAP and HR were 76±4 mmHg and 416±22 beats min^{-1} before GEA 3162 ($n=7$) and 79±4 mmHg and 401±23 beats min^{-1} before nitroprusside ($n=6$).

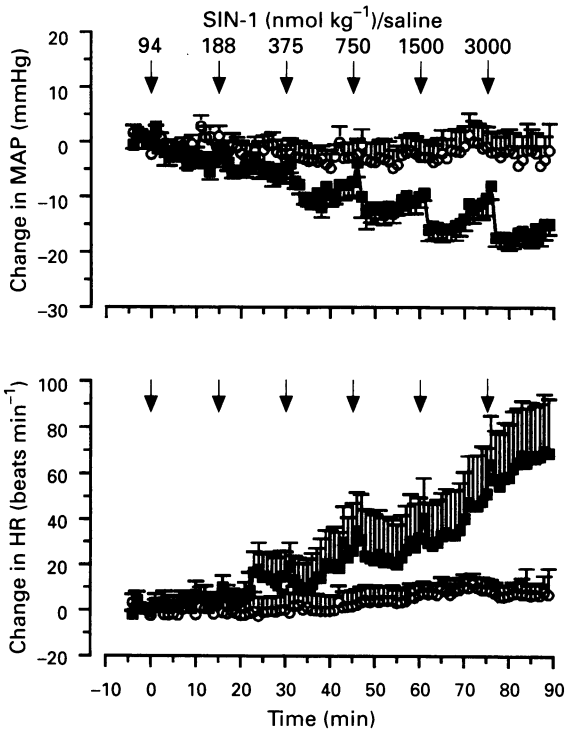

Figure 2 Time-course showing the effect of cumulative intravenous doses of SIN-1 (94–3000 nmol kg^{-1}; ■) or repeated injections of saline (0.1 ml; ○) over the same period of time on mean arterial pressure (MAP) and heart rate (HR). Initial baseline levels for MAP and HR were 78±2 mmHg and 365±13 beats min^{-1} before SIN-1 (n=6) and 83±3 mmHg and 387±26 beats min^{-1} before saline (n=7).

line value of 365±13 beats min^{-1}. The maximal reductions in blood pressure were obtained within 4–10 min while the increases in HR peaked within 5–12 min following SIN-1, i.v.

Repeated injections of physiological saline (0.1 ml, i.v.) at 10–15 min intervals had no significant effects on MAP or HR (P>0.05) (Figure 2).

Cardiovascular effects of NO-releasing substances after intracerebroventricular administration

Intracerebroventricularly administered GEA 3162 (24–188 nmol kg^{-1}) induced dose-dependent decreases in MAP (P<0.001) accompanied by slight and insignificant increases in HR (P=0.09) (Table 1). The time-course of the blood pressure responses to intravenous and intracerebroventricular GEA 3162 was similar, the maximal effect in MAP was observed within 30–60 s and blood pressure returned to the baseline level within 2 min following cumulative administrations of GEA 3162 i.c.v.

Cumulative intracerebroventricular injections of nitroprusside (24–188 nmol kg^{-1}) tended to decrease blood pressure with low doses, while MAP increased slightly after the largest dose. However, the changes in blood pressure were not significant when compared to the initial baseline value (Table 1). HR increased significantly after nitroprusside i.c.v. (P<0.001).

SIN-1 (94–3000 nmol kg^{-1}) produced minor increases in blood pressure upon intracerebroventricular administration (P<0.05). However, when compared to the initial baseline value, the increase in MAP was significant only after the largest dose of 3000 nmol kg^{-1}, i.c.v. (Table 1). There was a marked and dose-dependent tachycardia after cumulative intracerebroventricular administration of SIN-1 (P<0.001). The increase in HR relative to the initial baseline level was significant even with the lowest dose of SIN-1 used in the experiments (94 nmol kg^{-1}, i.c.v.).

Table 1 Maximal effects of cumulative intracerebroventricular injections of GEA 3162, nitroprusside and SIN-1 on mean arterial pressure (MAP) and heart rate (HR)

Dose (nmol kg^{-1})	n	*MAP (mmHg)*	*HR (beats min^{-1})*
GEA 3162			
Baseline	(6)	80±5	425±25
24		76±5*	437±24
47		71±6*	444±23
94		67±5**	438±22
188		58±5***	448±22
Nitroprusside			
Baseline	(6)	78±3	416±14
24		72±4	440±14*
47		73±4	446±12**
94		76±4	449±12**
188		88±3	455±7*
SIN-1			
Baseline	(6)	79±4	383±6
94		84±1	405±9*
188		82±4	421±10*
375		83±4	438±11*
750		87±3	462±13*
1500		92±3	478±10**
3000		91±2*	504±12**

Results are expressed as mean±s.e.mean from the number of experiments indicated in parentheses. Statistics: *P<0.05, **P<0.01, ***P<0.001 vs. corresponding baseline.

Repeated intracerebroventricular injections of saline at 10–15 min intervals had no significant effects on blood pressure or HR (P>0.05), the maximal changes in MAP and HR varied from 2±1 to 4±2 mmHg and from 10±8 to 13±7 beats min^{-1}, respectively (n=6).

Effect of pretreatment with methylene blue or LY-83583 on the cardiovascular responses to GEA 3162

Intravenously administered GEA 3162 (188 nmol kg^{-1}) reduced MAP from an initial value of 81±4 mmHg to 49±4 mmHg in saline pretreated animals (0.1 ml, i.v., n=4), whereas the corresponding decrease was from 75±4 mmHg to 52±3 mmHg after intravenous pretreatment with methylene blue (5 mg kg^{-1}, n=4). GEA 3162 i.v. increased HR from 423±26 beats min^{-1} to 462±23 beats min^{-1} and from 411±20 beats min^{-1} to 432±23 beats min^{-1} after intravenous saline and methylene blue, respectively. No significant differences in the baseline levels before GEA 3162 i.v. nor in the cardiovascular responses to the NO-donor between the two groups were observed.

Intracerebroventricular GEA 3162 (188 nmol kg^{-1}) decreased MAP from 82±3 mmHg to 58±2 mmHg after pretreatment with methylene blue (5 mg kg^{-1}, i.c.v., n=4) and from 81±5 mmHg to 62±4 mmHg after pretreatment with saline (10 μl, i.c.v., n=4). HR responses to GEA 3162 i.c.v. were from 433±12 beats min^{-1} to 479±16 beats min^{-1} and from 415±20 beats min^{-1} to 453±23 beats min^{-1} in the group pretreated with intracerebroventricular methylene blue and saline, respectively. There were no significant differences in the baseline levels nor in the cardiovascular responses to GEA 3162 i.c.v. between the two groups.

In preliminry experiments, we also studied the effects of intracerebroventricular pretreatment with LY-83583 (15 μg), an inhibitor of guanylate cyclase. The cardiovascular effects of GEA 3162 i.c.v. were unaltered after pretreatment with this substance.

Discussion

In the present study nitroprusside, GEA 3162 and SIN-1, substances which generate NO, dose-dependently decreased

blood pressure after intravenous administration. The hypotensive effects after nitroprusside and GEA 3162 were similar. The onset was rapid and the duration of action was short-lasting, which is most likely related to the fast NO release from the molecules and extremely short half-life of NO (Moncada *et al.*, 1991; Karup *et al.*, 1994). Intravenously administered SIN-1 was a less potent and less effective hypotensive agent than the other NO-releasing drugs used in our experiments. Also *in vitro* studies with these substances in rabbit aortic strips showed that the vasorelaxant potency of SIN-1 was 20–70 times less than that of nitroprusside or GEA 3162 (Corell *et al.*, 1994). Furthermore, the onset of the action was slower after SIN-1 than after the other drugs, which is in agreement with the slow NO release from SIN-1 (Feelisch *et al.*, 1989).

GEA 3162 also produced a dose-related hypotensive effect after intracerebroventricular administration. The immediate onset of the effect suggest a central site of action. The decrease in blood pressure following the NO-releasing substance is in line with earlier studies which demonstrated that inhibition of endogenous NO formation in the brain by centrally administered NO synthase inhibitors increased blood pressure (Togashi *et al.*, 1992; Cabrera & Bohr, 1995). Therefore, it seems that NO, acting centrally, causes a depressor response, and withdrawal of endogenous NO tone leads to elevation of blood pressure.

In our study, intracerebroventricular nitroprusside had no significant effect on blood pressure at the same doses which were hypotensive when given peripherally. Only minor cardiovascular responses after intracerebroventricular nitroprusside in anaesthetized rats have also been observed by other groups (Ma & Long, 1992), while in a recent study intracerebroventricular nitroprusside produced a decrease in blood pressure with a moderate rise in heart rate in anaesthetized cats (Hedge *et al.*, 1994). The effects of nitroprusside were abolished by spinal cord transection indicating that they were central in origin. On the other hand, another NO-donor, SNAP, injected into the lateral cerebral ventricle has been reported to increase blood pressure in conscious rats (Ota *et al.*, 1993). In our study, the largest dose of nitroprusside slightly but not significantly increased blood pressure, while a significant increase in blood pressure was observed after a large intracerebroventricular dose of SIN-1. Therefore, it is possible that NO has different effects on blood pressure depending on the dose. Exogenously administered NO may exert a hypotensive effect with low doses, whereas large doses produce elevation of blood pressure.

The different blood pressure responses to the NO donors observed in this study may also reflect different accessibility of various sites within the CNS, when given intracerebroventricularly. Microinjections of nitroprusside into the ventrolateral medulla of cat have been shown to increase or decrease blood pressure depending on the injection site (Shapoval *et al.*, 1991). Furthermore, locally microinjected nitroprusside or NO-containing artificial cerebrospinal fluid into the hypothalamic paraventricular nucleus decreased blood pressure in anaesthetized rats (Horn *et al.*, 1994). The lack of hypotensive effect after intracerebroventricular SIN-1 and nitroprusside in our study may be due to the relative poor ability of these substances to penetrate cell membranes (Southam & Garthwaite, 1991).

Heart rate increased concomitantly with the hypotensive response to systemically administered NO-releasing substances. The tachycardia may be a result of withdrawal of vagal tone or reflex activation of the sympathetic nervous system due to the hypotensive action of these drugs. However, SIN-1 increased heart rate even in doses which did not markedly affect blood pressure. Thus, other mechanisms may also be involved in the tachycardia induced by NO-donors. It has been suggested that the tachycardic response to intravenous organic nitrates may be mediated, at least partly, through a direct central action (Ma & Long, 1992). It is noteworthy that heart rate increased after central administrations of nitroprusside and SIN-1 at doses which did not exert a significant effect on blood pressure. There was also a slight but insignificant increase in heart rate after centrally injected GEA 3162. The increase in heart rate after these NO-releasing compounds suggests that exogenously administered NO may represent the active factor underlying the effect.

A principal mechanism by which NO exerts many of its biological or pharmacological actions is via stimulation of the soluble guanylate cyclase with subsequent increase in the intracellular levels of cyclic GMP in target cells (Moncada *et al.*, 1991). This may also be the fact in the CNS, since nitroprusside and SIN-1 have been demonstrated to elevate cyclic GMP levels in rat cerebellar slices (Southam & Garthwaite, 1991). Also pharmacological effects of GEA 3162 were associated with a rise in cellular cyclic GMP in peripheral tissues and cells (Corell *et al.*, 1994; Kankaanranta *et al.*, 1996). Methylene blue is considered as an inhibitor of the NO-stimulated soluble guanylate cyclase and has been widely used for suppression of the activation of the cyclic GMP-mediated processes by nitrovasodilators (Gryglewski *et al.*, 1992). In a recent study with anaesthetized cats, the hypotensive effect following intracerebroventricular nitroprusside was attenuated by methylene blue (Hedge *et al.*, 1994). However, in our study the cardiovascular responses to peripherally or centrally administered GEA 3162 were not antagonized by pretreatment with methylene blue. Furthermore, in preliminary experiments the cardiovascular effects of GEA 3162 were not susceptible to blockade by LY-83583, a substance which lowers cyclic GMP levels in a wide range of tissues (Mülsch *et al.*, 1988). Based on our results it seems that the cardiovascular effects of GEA 3162 are mediated at least partially through a yet unknown mechanism independent of guanylate cyclase. However, this does not rule out the possibility that NO released from the substance is underlying the effects obtained. A number of effects of NO have recently been reported to be mediated by mechanisms that are not related to the stimulation of soluble guanylate cyclase and the production of cyclic GMP. For example, NO can directly activate calcium-dependent potassium channels in vascular smooth muscle (Bolotina *et al.*, 1994). NO-generating agents have been demonstrated to cause an activation of ADP-ribosyltransferase and an inactivation of glyceraldehyde-3-phosphate dehydrogenase by a cyclic GMP-independent mechanism (Brune & Lapetina, 1989; Molina y Vedia *et al.*, 1992). NO also stimulates the activity of Na^+-K^+-ATPase in blood vessels through a pathway that does not involve guanylate cyclase (Gupta *et al.*, 1994). Furthermore, NO regulates NMDA receptors via S-nitrosylation of free thiol groups (Lipton *et al.*, 1993).

In summary, intravenously administered NO-donors, nitroprusside and GEA 3162, induced a dose-dependent and short lasting hypotensive effect accompanied by a transient tachycardia. Systemically administered SIN-1 lowered blood pressure only weakly, while the tachycardic effect after SIN-1 was prominent and long-lasting. Centrally administered GEA 3162 induced a dose-dependent, marked hypotensive response without significant changes in heart rate. In contrast, intracerebroventricular injections of nitroprusside and SIN-1 increased heart rate without affecting blood pressure. The differences in the responses may be related to different pharmacokinetic profiles and/or different time course of the NO release from the compounds. Our findings suggest that NO released from these drugs affects central cardiovascular regulation.

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