J Neural Transm (2007) 114: 863–865
DOI 10.1007/s00702-007-0702-z
Printed in the Netherlands

Journal of Neural Transmission

Hypotensive effect of hydroxylamine, an endogenous nitric oxide donor and SSAO inhibitor

H. Vidrio, M. Medina

Department of Pharmacology, Faculty of Medicine, Universidad Nacional Autónoma de México, Mexico City, Mexico

Received: September 9, 2006 / Accepted: October 13, 2006 / Published online: March 26, 2007

Summary The endogenous compound hydroxylamine relaxes vascular smooth muscle *in vitro*, apparently through conversion to the vasodilator factor nitric oxide, but its effect on blood pressure has not been characterized. We found that in the anesthetized rat the amine elicits dose-related hypotension when administered by continuous iv infusion. In experiments designed to explore the mechanism of this effect, hydroxylamine was compared with the nitric oxide donor nitroprusside and the direct-acting vasodilator hydralazine, using pretreatments known to modify diverse mechanisms of vasodilation. Hydroxylamine hypotension was enhanced by the SSAO inhibitor isoniazid and the SSAO substrate methylamine, a pattern shared by hydralazine. Responses were blocked by the guanylate cyclase inhibitor methylene blue and were increased by the nitric oxide synthase inhibitor L-NAME, a pattern shared by nitroprusside. It was concluded that hydroxylamine exerts hypotension partly through conversion to nitric oxide and partly by a "hydralazine-like" mechanism involving SSAO inhibition.

Keywords: Hydroxylamine, nitroprusside, hydralazine, nitric oxide, SSAO

Introduction

Hydroxylamine is an endogenous compound produced by normal mammalian cell metabolism and formed by reduction of nitrates or nitrites or by oxidation of ammonia (Gross, 1985). The physiological importance of hydroxylamine rests in its conversion to the vasodilator transmitter nitric oxide, a reaction shown to occur *in vitro* (Taira et al., 1997; Klink et al., 2001). As a consequence of this conversion, the amine activates guanylate cyclase and induces vascular relaxation *in vitro* (Rapoport and Murad, 1984). Although it produces transient hypotension in anesthetized dogs and cats (Kruszyna et al., 1984), this response has not been completely characterized.

Correspondence: Horacio Vidrio, M.D., Department of Pharmacology, Faculty of Medicine, Universidad Nacional Autónoma de México, Apartado Postal 70297, 04510 México, D.F., México
e-mail: vidrio@servidor.unam.mx

On the other hand, hydroxylamine inhibits the activity of the enzyme generally known as semicarbazide-sensitive amine oxidase (SSAO) (Lyles and Singh, 1985). Although the physiological role of this enzyme has not been unequivocally established, we have postulated its possible influence on vascular tone, through generation of the vasoactive product H_2O_2 (Vidrio, 2003). Since SSAO is particularly abundant in vascular smooth muscle (Lyles and Singh, 1985) and since H_2O_2 at physiological concentrations produces vasoconstriction (Gil-Longo and González-Vázquez, 2005), this product could function as a locally released constrictor factor. The hypothesis, mainly based on studies with hydralazine, a potent peripheral vasodilator and SSAO inhibitor, also postulates that SSAO inhibition could interrupt this process, leading to vasodilation and hypotension.

Based on the above consideratioons, the present study was carried out to determine whether the mechanisms mentioned, conversion to nitric oxide and/or SSAO inhibition, could be involved in the hypotensive effect of hydroxylamine. To this end, blood pressure responses to the amine were obtained in control anesthetized rats and compared to those of animals subjected to various pretreatments designed to selectively influence these mechanisms. Results with hydroxylamine were compared to those with the nitric oxide donor sodium nitroprusside and with the SSAO inhibitor hydralazine.

Material and methods

Male Wistar rats weighing between 200 and 300 g were anesthetized with chloralose, 50 mg/kg, and urethane, 750 mg/kg, both administered ip.

Cannulas were inserted in a femoral artery and vein for blood pressure recording and drug administration, respectively. Mean blood pressure was recorded continuously with a transducer and polygraph system. In a first series of experiments, hypotensive responses to three dose levels of hydroxylamine, nitroprusside and hydralazine were obtained in control unpretreated rats. Since the first two agents produced transient responses when injected as a bolus, they were administered by 60 min iv infusions; hydralazine was give as a bolus and the response recorded for 60 min thereafter. The doses tested were hydroxylamine, 25, 200 and 500 μg/kg/min; nitroprusside, 2, 10 and 25 μg/kg/min; hydralazine, 100, 320 and 560 μg/kg. These doses produced threshold, intermediate and maximum hypotensive responses of approximately equal magnitude.

In a second experimental series, rats receiving threshold doses of the hypotensive agents were pretreated with the SSAO substrate methylamine or the SSAO inhibitor isoniazid; other animals receiving intermediate doses were pretreated with the enzyme inhibitors methylene blue (guanylate cyclase) or L-NAME (nitric oxide synthase). Pretreatment schedules and doses were as follows: methylamine, 200 mg/kg ip 2 h previously; isoniazid, 30 mg/kg ip 30 min; methylene blue, 31 μg/kg/min iv; L-NAME, 50 μg/kg/min. The last two infusions were started respectively 30 and 90 min before administration of the test hypotensive agents and were continued for 60 min thereafter.

All drugs were dissolved in isotonic NaCl solution; those administered by infusion were delivered in a volume of 0.05 ml/min. In the case of nitroprusside, the infusion syringe was protected from light. Hypotensive responses were expressed as mean blood pressure decreases ± SEM, calculated by determining areas under the curve for individual experiments. Responses in pretreated groups were compared to corresponding controls by one-way ANOVA and Dunnett's test; a probability of less than 5% was considered as indicating significance.

Results

In control animals, hydroxylamine decreased blood pressure by 10 ± 2, 20 ± 2 and 52 ± 2 mmHg for 60 min when administered at low, intermediate and high doses. Corresponding figures were 14 ± 2, 31 ± 3 and 48 ± 3 for nitroprusside and 14 ± 2, 38 ± 4 and 48 ± 2 for hydralazine.

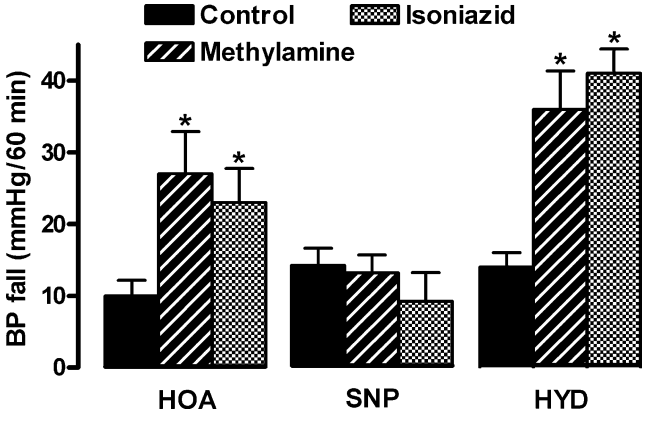

Fig. 1. Influence of methylamine and isoniazid on hypotension elicited by low doses of hydroxylamine (*HOA*), sodium nitroprusside (*SNP*) and hydralazine (*HYD*). Bars represent mean blood pressure falls over 60 min during (HOA, SNP) or after (HYD) administration of the hypotensive agents. Shown are standard errors (vertical lines) and significant differences from control (asterisks)

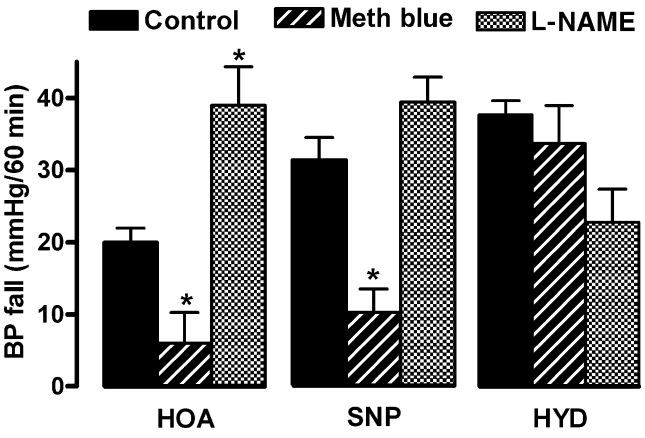

Fig. 2. Influence of methylene blue and L-NAME on hypotension elicited by intermediate doses of hydroxylamine (*HOA*), sodium nitroprusside (*SNP*) and hydralazine (*HYD*). Bars represent mean blood pressure falls over 60 min during (HOA, SNP) or after (HYD) administration of the hypotensive agents. Shown are standard errors (vertical lines) and significant differences from control (asterisks)

Methylamine and isoniazid increased responses to hydroxylamine and hydralazine, but did not affect those to nitroprusside (Fig. 1). Methylene blue decreased responses to hydroxylamine and nitroprusside, whereas L-NAME increased the effect of hydroxylamine only. Responses to hydralazine were nor affected by these pretreatments (Fig. 2).

Discussion

The present results suggest that in the rat hydroxylamine produces hypotension by behaving as a nitric oxide donor, thus confirming previous *in vitro* observations. Blockade of hypotensive responses by methylene blue indicate mediation by guanylate cyclase activation, as reported *in vitro* (Rapoport and Murad, 1984). Potentiation of responses by L-NAME is compatible with an effect exerted by conversion to nitric oxide independently of nitric oxide synthase. In this respect, L-NAME is known to increase vascular smooth muscle relaxation by direct nitric oxide donors, a phenomenon attributed to a supersensitivity of guanylate cyclase in the absence of endogenous nitric oxide (Moncada et al., 1991). It should be noted that responses to nitroprusside were also inhibited by methylene blue and potentiated by L-NAME, although the latter effect did not reach statistical significance.

The results also indicate that hydroxylamine resembles hydralazine in its mechanism of hypotensive activity, since responses to both drugs were potentiated by isoniazid and methylamine. Previous studies from this laboratory show that isoniazid selectively enhances hydralazine hypotension

(Vidrio et al., 2002). This interaction was attributed to the fact that both isoniazid (Lewinsohn et al., 1978) and hydralazine (Lyles et al., 1983) are potent SSAO inhibitors and was taken as evidence for a link between hydralazine hypotension and enzymatic inhibition. The finding that responses to hydralazine are also enhanced by methylamine, the purported endogenous SSAO substrate (Vidrio et al., 2003), further supports this hypothesis. Thus, hydroxylamine, which is also a potent SSAO inhibitor (Lyles and Singh, 1985), could be acting in part by this mechanism to produce vasodilation and hypotension.

In conclusion, the present results show that the endogenous compound hydroxylamine elicits hypotension in the rat, and that this effect is due in part to its conversion to nitric oxide and in part to a ''hydralazine-like'' action involving SSAO inhibition.

References

Gil-Longo J, González-Vázquez C (2005) Characterization of four different effects elicited by H_2O_2 in rat aorta. Vascul Pharmacol 43: 128–138

Gross P (1985) Biologic activity of hydroxylamine: a review. Crit Rev Toxicol 14: 87–99

Klink M, Swierzko A, Sulowska Z (2001) Nitric oxide generation from hydroxylamine in the presence of neutrophils and in the cell-free system. APMIS 109: 493–499

Kruszyna R, Kruszyna H, Doherty PA, Smith RP (1984) Hypotensive effects of hydroxylamine in intact anesthetized dogs and cats. Arch Toxicol 55: 203–205

Lewinsohn R, Böhm KH, Glover V, Sandler M (1978) A benzylamine oxidase distinct from monoamine oxidase B – widespread distribution in man and rat. Biochem Pharmacol 27: 1857–1863

Lyles GA, Singh I (1985) Vascular smooth muscle cells: a major source of the semicarbazide-sensitive amine oxidase of the rat aorta. J Pharm Pharmacol 37: 637–643

Lyles GA, García-Rodríguez J, Callingham BA (1983) Inhibitory actions of hydralazine upon monoamine oxidizing enzymes in the rat. Biochem Pharmacol 32: 2515–2521

Moncada S, Rees DD, Schulz R, Palmer RMJ (1991) Development and mechanism of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis *in vivo*. Proc Natl Acad Sci USA 88: 2166–2170

Rapoport RM, Murad F (1984) Effect of cyanide on nitrovasodilator-induced relaxation, cyclic GMP accumulation and guanylate cyclase activation in rat aorta. Eur J Pharmacol 104: 61–70

Taira J, Misik V, Riesz P (1997) Nitric oxide formation from hydroxylamine by myoglobin and hydrogen peroxide. Biochem Biophys Acta 1336: 502–508

Vidrio H (2003) Semicarbazide-sensitive amine oxidase: role in the vasculature and vasodilation after *in situ* inhibition. Auton Autacoid Pharmacol 23: 275–283

Vidrio H, Medina M, Fernández G, Lorenzana-Jiménez M, Campos AE (2002) Enhancement of hydralazine hypotension by low doses of isoniazid. Possible role of semicarbazide-sensitive amine oxidase inhibition. Gen Pharmacol 35: 195–204

Vidrio H, Medina M, González-Romo P, Lorenzana-Jiménez M, Díaz-Arista P, Baeza A (2003) Semicarbazide-sensitive amine oxidase substrates potentiate hydralazine hypotension: possible role of hydrogen peroxide. J Pharmacol Exp Ther 307: 497–504