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Pharmacological evidence that NaHS inhibits the vasopressor responses induced by stimulation of the preganglionic sympathetic outflow in pithed rats

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ABSTRACT

It has been reported that i.v. administration of NaHS, a donor of H_2S, elicited dose-dependent hypotension although the mechanisms are not completely understood. In this regard, several mechanisms could be involved including the inhibition of the vasopressor sympathetic outflow. Thus, this study was designed to determine the potential capability of NaHS to mediate inhibition of the vasopressor responses induced by preganglionic sympathetic stimulation. For this purpose, Wistar rats were anaesthetised, pithed and cannulated for drug administration. In animals pre-treated with gallamine, the effect of i.v. infusion of NaHS (310 and 560 μg/kg min) or its vehicle (phosphate buffer) was determined on the vasopressor responses induced by: (1) sympathetic stimulation (0.03–10 Hz); (2) i.v. bolus injections of exogenous noradrenaline (0.03–3 μg/kg); or (3) methoxamine (1–100 μg/kg). The vasopressor responses induced by preganglionic sympathetic stimulation were dose-dependently inhibited by i.v. infusion of NaHS (310 and 560 μg/kg min), but not by vehicle, particularly at high frequencies. In marked contrast, the vasopressor responses to exogenous noradrenaline or methoxamine were not inhibited by the above doses of NaHS or its vehicle. The above results, taken together, demonstrate that NaHS inhibited the vasopressor responses induced by preganglionic sympathetic outflow by a prejunctional mechanism. This is the first evidence demonstrating this effect by NaHS that may contribute, at least in part, to the hypotension induced by NaHS.

1. Introduction

H_2S is a gasotransmitter that mediates complex responses in the cardiovascular system such as cardioprotection and vasculoprotection (Wang, 2012). In isolated blood vessels, H_2S produces relaxation mainly by activation of K_{ATP} channels (Tang et al., 2010) while in anaesthetised rats NaHS, a donor of H_2S, mediates hypotension (Ali et al., 2006) although the mechanisms are more complex and several unidentified mechanisms could be involved. Indeed, in anaesthetised rats, hypotension to NaHS is not mediated by nitric oxide release, K+ channels, BKCa channels, cGMP, release of arachidonic acid metabolites or p450 epoxygenase metabolites. This effect was not mediated by adrenergic or cholinergic system since the hypotension to NaHS was unaffected by atropine, phentholamine or hexamethonium (Yoo et al., 2015). Thus, the authors concluded that uncertain mechanisms mediate hypotension to NaHS in anaesthetised rats.

One of the mechanisms that we explore in this study is the capability of NaHS to inhibit the vascular sympathetic outflow. In this context, it has been previously shown that NaHS inhibited [H^3]-noradrenaline release elicited by electrically field stimulation in the sympathetic porcine iris-ciliary bodies (Kulkarni et al., 2009). The enzyme inhibitors propargylglycine (CSE) and aminooxyacetic acid (CBS) blocked this effect suggesting that this effect was dependent on intramural biosynthesis of H_2S.

On this basis, the present study was designed to determine the potential capability of NaHS to inhibit the vasopressor sympathetic outflow in pithed rats. This mechanism may be involved in the vasodepressor effect induced by NaHS.

2. Materials and methods

2.1. Animals

Male Wistar normotensive rats (270–300 g) were housed in plastic cages in a special temperature-controlled room (22 ± 2 °C, 50% humidity) on a 12/12-h light-dark cycle (with light beginning at 7:00 a.m.), with food and water freely available in their home

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cages. All animal procedures and the protocols of the present investigation were approved by our Institutional Ethics Committee (Cicual-Cinvestav), and followed the regulations established by the Mexican Official Norm for the Use and Welfare of Laboratory Animals (NOM-062-ZOO-1999), in accordance with the Guide for the Care and Use of Laboratory Animals in U.S.A.

2.2. General procedure

The animals were anaesthetised with isoflurane (3%). The trachea was cannulated and the rats were pithed by inserting a stainless-steel rod through the orbit and *foramen magnum* into the vertebral *foramen* (Gillespie and Muir, 1967). Under these conditions, the animals were unconscious as the central nervous system was destroyed (Centurión et al., 2009). Then, the animals were artificially ventilated with room air using a positive pressure pump (7025 rodent ventilator, Ugo Basile, Comerio, VA, Italy) at 56 strokes/min and a stroke volume of 20 ml/kg, as previously established (Kleinman and Radford, 1964). After bilateral vagotomy, catheters were placed in: (i) the left and the right femoral veins for drugs administration and (ii) the left carotid artery. The latter was connected to a pressure transducer (P23 XL, Grass Technologies, Warwick, RI, U.S.A.) to record the arterial blood pressure and heart rate. Blood pressure and heart rate were recorded simultaneously using a data acquisition unit (MP150A-CE, Biopac Systems Inc., Goleta, CA) and Acknowledge software v3.8.1 (Biopac Systems Inc., Goleta, CA). Diastolic blood pressure was determined, as this is the blood pressure when the left ventricle is relaxed and thus could indirectly represent the systemic vascular resistance that regulates arterial blood pressure and blood flow within organs.

2.3. Experimental protocol

After this procedure, the 54 rats were initially divided into two main sets, so that the vasopressor responses produced by: (i) selective preganglionic (T7-T9) stimulation of the vasopressor sympathetic outflow (set 1, $n=18$); or (ii) i.v. bolus injections of exogenous noradrenaline or methoxamine (set 2; $n=36$) were investigated under different treatments (see experimental protocols below). The vasopressor stimulus-response or dose-response curves elicited by, respectively, sympathetic stimulation or exogenous noradrenaline and methoxamine were completed in about 45 min. Moreover, the vasopressor sympathetic stimuli (0.03, 0.1, 0.3, 1, 3 and 10 Hz) as well as i.v. bolus injections of noradrenaline (0.03, 0.1, 0.3, 1, and 3 μg/kg) or methoxamine (1, 3, 10, 30 and 100 μg/kg) were given using a sequential schedule of 0.5 log unit increments. The interval between the different stimulation frequencies/doses of noradrenaline and methoxamine was dependent on the duration of the resulting vasopressor responses (5–10 min.), as we waited until diastolic blood pressure had returned to baseline values. The body of each pithed rat was maintained at 37 °C by a lamp and monitored with a rectal thermometer.

2.3.1. Protocol 1. Stimulation of the vasopressor sympathetic outflow

In the first set of rats ($n=18$), the stainless-steel rod was replaced by an enamelled electrode except for 1 cm length 9 cm from the tip. The uncovered segment was situated at the T7-T9 region of the spinal cord to allow for selective stimulation of the sympathetic nerves supplying the systemic vasculature (Gillespie et al., 1970; Monroy-Ordoñez et al., 2008). Prior to electrical stimulation, the animals received gallamine (25 mg/kg, i.v.) to avoid electrically-induced muscular twitching. After a stable hemodynamic condition was achieved lasting at least 15 min, baseline values of diastolic blood pressure and heart rate were determined. Then, the preganglionic vasopressor sympathetic outflow was stimulated with a S88X square pulse stimulator (Grass Technologies, Warwick, RI, U.S.A.) by applying 10 s trains of monophasic, rectangular pulses (2 ms, 50 V), at increasing frequencies of stimulation (0.03, 0.1, 0.3, 1.0, 3.0 and 10 Hz). An SIU-V isolation unit (Grass Technologies, Warwick, RI, U.S.A.) was used to minimise artefacts resulting from the stimuli. When blood pressure returned to baseline levels, the next frequency was applied; this procedure was performed systematically until the stimulus-response curve was completed (about 45 min).

Then, the first set ($n=18$) was divided into three subgroups ($n=6$ each) that received, by a WPI model sp100i pump (World Precision Instruments Inc., Sarasota, FL, U.S.A.), i.v. continuous infusions of, respectively: (i) phosphate buffer saline (0.02 ml/min); (ii) NaHS (310 μg/kg min); and (iii) NaHS (560 μg/kg min). Twenty minutes later, a stimulus-response curve was constructed again during the infusion of the above compounds to analyse their effects on the sympathetically-induced vasopressor responses. Once the stimulus-response curve had been completed, the infusion was stopped. The doses of NaHS were chosen from preliminary experiments in which lower doses than 310 μg/kg min produced no effect on sympathetic stimulation while higher doses than 560 μg/kg min produced toxic effects.

Table 1
Effect of i.v. infusion of vehicle (phosphate buffer saline) or NaHS on diastolic blood pressure and heart rate.

Treatment	Dose (μg/kg min)	Diastolic Blood Pressure (mmHg)		Heart Rate (beats/min)	
		Before	10 min after	Before	10 min after
Vehicle	0.02[a]	25 ± 3	23 ± 3	301 ± 9	285 ± 10
NaHS	310	23 ± 1	25 ± 2	340 ± 14	312 ± 12
NaHS	560	23 ± 2	21 ± 2	342 ± 54	294 ± 43

[a] ml/min.

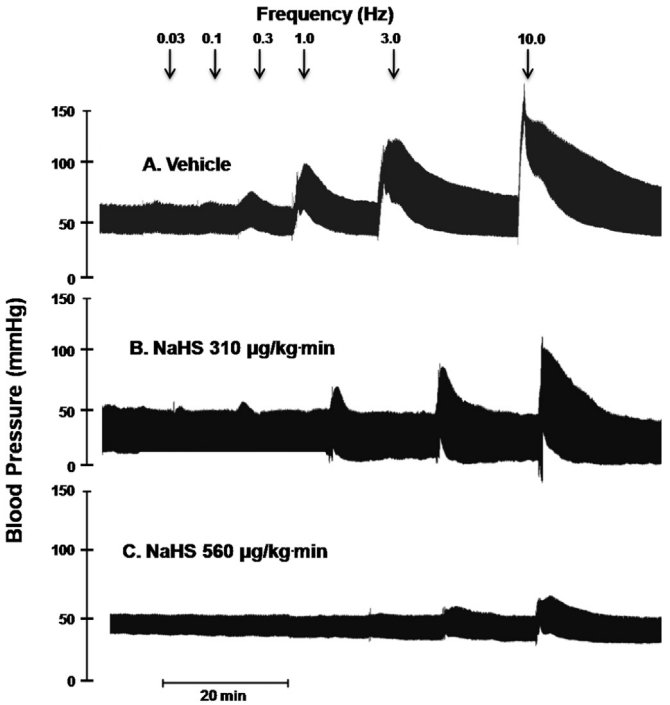

Fig. 1. Original recordings showing the vasopressor responses induced by electrical stimulation of the vasopressor sympathetic outflow at the frequencies of 0.03–3 Hz in presence of i.v. infusion of: (A) vehicle (phosphate buffer saline, 0.02 ml/min); (B) 310 μg/kg min NaHS; and (C) 560 μg/kg min NaHS.