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NaHS prejunctionally inhibits the cardioaccelerator sympathetic outflow in pithed rats

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ARTICLE INFO

Compounds:
Gallamine triethiodide (PubChem ID: 6172)
(±)-noradrenaline bitartrate (PubChem ID: 297812)
Isoproterenol hydrochloride (PubChem ID: 5807)
Sodium hydrosulfide monohydrate (PubChem ID: 28015)

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ABSTRACT

Hydrogen sulfide is a gasotransmitter that mediates cardiovascular responses and could protect the heart from ischemia-reperfusion damage. Furthermore, this gas mediates bradycardia although the mechanisms involved remain elusive. In this regard, the inhibition of the cardiac sympathetic outflow may be partially involved. Thus, this study was designed to determine the capability of NaHS to inhibit the tachycardic responses induced by preganglionic stimulation of the cardioaccelerator sympathetic outflow. Wistar rats were anaesthetized with isoflurane, cannulated and pithed. Then, animals received gallamine and the effect of i.v. infusion of NaHS (310 and 560 μg/kg min) was evaluated on the tachycardic responses induced by (1) sympathetic stimulation (0.1–3.2 Hz) at C_7-T_1 region of the vertebral column; or i.v. injections of (2) noradrenaline (0.03–3 μg/kg) and (3) isoproterenol (0.0003–0.1 μg/kg). Notably, NaHS significantly and dose-dependently inhibited the tachycardic responses induced by electrical stimulation of the preganglionic sympathetic outflow without significantly modify the tachycardic responses induced by either noradrenaline or isoproterenol. These results allow us to conclude that i.v. infusion of NaHS inhibited the tachycardic responses induced by stimulation of the cardioaccelerator sympathetic outflow by a prejunctional mechanism.

1. Introduction

In the last 20 years, the physiological and pathophysiological role of hydrogen sulfide in the cardiovascular system has been explored in several experimental preparations. As a result, it has been demonstrated that this gas produces vasodilatation (Hosoki et al., 1997; Zhao et al., 2001), vasoconstriction (Lim et al., 2008), hypotension (Ali et al., 2006), hypertension (Ufnal et al., 2008) and negative chronotropic, inotropic and dromotropic effects (Geng et al., 2004).

Concerning the cardiac effects of H_2S, it has been demonstrated that in the rat isolated heart, NaHS, an H_2S donor, produced negative inotropic and chronotropic responses (Geng et al., 2004; Porokhya et al., 2012). Indeed, in isolated and perfused Langendorff rat heart, NaHS produced: (1) decrease of (±)-LV dp/d_{tmax} at $10^{-6}-10^{-9}$ mol/L which suggests a negative inotropic response (Geng et al., 2004); (2) negative lusitropism (Mazza et al., 2013); and (3) negative chronotropism at higher concentrations of NaHS (10^{-3} mol/L) (Geng et al., 2004). Unfortunately, the mechanisms involved in the cardiac effects remain elusive. In an effort to elucidate the mechanisms it has been shown that: (1) glibenclamide partially blocked the negative inotropism induced by NaHS, suggesting that K_{ATP} could be involved in this effect (Chen et al., 2012; Xu et al., 2008); (2) 10^{-9} M NaHS increased phosphorylation of Akt (Ser-493) and eNOS (Ser-1177) in rat heart implying that activation of this pathway may be involved in the negative inotropic effect of NaHS (Mazza et al., 2013); and (3) NaHS inhibited isoproterenol-induced calcium transient by L-type calcium channel (Sun et al., 2008; Zhang et al., 2012) and Serca2 associated with phospholamban (Chen et al., 2012). Consistent with the above, cystathionine-γ−lyase (CSE) mRNA was found in the rat heart (Fu et al., 2012).

On the other hand, in anaesthetized rats, i.v. administration of NaHS produced dose-dependent bradycardia although this effect was not mediated by nitric oxide release, K^+ channels, BK_{Ca} channels, cGMP, the release of arachidonic acid metabolites or p450 epoxygenase metabolites. Moreover, atropine, phentolamine or hexamethonium did not affect NaHS-induced bradycardia, which may suggest that this response was not mediated by the adrenergic or cholinergic system (Yoo et al., 2015). Thus, the authors concluded that uncertain mechanisms mediate bradycardia to NaHS in anaesthetized rats. Interestingly, in anaesthetized rats with cardiac pacing, Na_2S-induced bradycardia was

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abolished (Swan et al., 2017). Despite the above findings, further data are needed to understand the cardiac effects of H_2S. Sympathetic tone is a key regulator of cardiac function as activation mediates positive inotropic, chronotropic and dromotropic responses. We have previously demonstrated that i.v. infusion of NaHS inhibited the vasopressor sympathetic outflow in the pithed rat (Centurión et al., 2016), a mechanism that could play a role in the regulation of the cardiovascular system. Therefore, the objective of this study was to evaluate the capability of NaHS to inhibit the electrically-induced tachycardic responses in pithed rats.

2. Materials and methods

2.1. Animals

Male Wistar normotensive rats (270–300 g) were used in the present experiments. The animals were maintained at a 12/12-h light-dark cycle (with light starting at 7:00 a.m.) and lodged in an especial room at constant temperature (22 ± 2 °C, 50% humidity), with water and food ad libitum in their acrylic cages. All methods and protocols of the current investigation were approved by our Institutional Ethics Committee (Cicual-Cinvestav), and followed the regulations accepted by the Mexican Official Norm for the Use and Welfare of Laboratory Animals (NOM-062-ZOO-1999), in compliance with the Guide for the Care and Use of Laboratory Animals in U.S.A (2011).

2.2. General methods

Experiments were carried out in a total of 72 rats. These animals were anesthetized with isoflurane (3%) and, subsequently, the trachea was cannulated to artificially ventilate the animals. Next, rats were pithed by inserting a stainless-steel stylet across the ocular orbit and *foramen magnum* into the vertebral foramen (Shipley and Tilden, 1947), in order to destroy the central nervous system and exclude central mechanisms that regulate blood pressure and heart rate (Centurión et al., 2009). The animals were artificially ventilated with room air using a Ugo Basile pump (7025 rodent ventilator, Comercio, VA Italy) at 56 strokes/min and stroke volume of 20 ml/kg (Kleinman and Radford, 1964). Subsequently, the pithing stylet was replaced by an electrode, which was isolated except for 1 cm length 7 cm from the tip, so the uncover segment was situated at C_7-T_1 region of the spinal cord to enable selective stimulation of the cardiac sympathetic outflow (Cobos-Puc et al., 2007). A bilateral vagotomy was made. Then, catheters were placed in: (1) the left femoral vein for NaHS continuous infusion; (2) the right femoral vein for drugs administration, such as gallamine, noradrenaline or isoproterenol; and (3) in the left carotid artery, which was connected to a pressure transducer (RX104A, Biopac Systems, Inc., Goleta, CA), for recording blood pressure and heart rate. Both haemodynamic parameters were recorded simultaneously using a data acquisition unit (MP150A-CE, Biopac Systems Inc., Goleta, CA) and AcqKnowledge software v3.8.1 (Biopac Systems Inc., Goleta, CA). The preganglionic cardioaccelerator sympathetic outflow was stimulated with an S88X square pulse stimulator (Grass Technologies, Warwick, RI, U.S.A.). Moreover, an SIU-V isolation unit (Grass Technologies, Warwick, RI, U.S.A.) was used to minimize artifacts resulting from the stimuli. Before electrical stimulation, animals received gallamine (25 mg/kg, i.v.) to avoid muscular twitching due to electrical stimulation. The body of each pithed rat was maintained at 37 °C using an incandescent lamp and monitored with a rectal thermometer.

2.3. Experimental protocol

After a stable haemodynamic condition for at least 15 min, baseline values of diastolic blood pressure and heart rate were determined. Then, the preganglionic cardiac sympathetic outflow was stimulated to elicit tachycardic responses by applying trains of 10 s (monophasic rectangular pulses of 2 ms duration and 60 V), at increasing frequencies of stimulation (0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 Hz). When heart rate had returned to baseline, the next frequency was applied; this procedure was systematically performed until the stimulus-response curve was completed (about 45 min). At this point, the animals (72 in total) were divided into two main sets: (1) cardioaccelerator sympathetic outflow stimulation (n = 24); or (2) i.v. bolus injections of either noradrenaline or isoproterenol (n = 48). Those responses were investigated under different treatments (see experimental protocols below). The dose-response curve elicited by, respectively, noradrenaline or isoproterenol was completed in about 60 min. Moreover, the i.v. bolus injections of noradrenaline (0.03, 0.1, 0.3, 1 and 3 μg/kg) and isoproterenol (0.0003, 0.001, 0.003, 0.01, 0.03 and 0.1 μg/kg) were given using a sequential schedule of 0.5 log unit increments. The time interval between the different doses of noradrenaline or isoproterenol was dependent on the duration of the resulting tachycardic responses (5–10 min), as we waited until heart rate had returned to baseline values.

2.3.1. Protocol 1. Stimulation of the cardioaccelerator sympathetic outflow

The first group of rats (n = 24) was divided into four subgroups (n = 6 each). The first subgroup: (1) was used to evaluate the tachycardic responses reproducibility and did not receive an infusion. All other subgroups received, by a WPI model sp100i pump (World Precision Instruments Inc., Sarasota, FL, U.S.A.), an i.v. continuous infusion of, respectively: (2) phosphate buffer saline (PBS; 0.02 ml/min; vehicle of NaHS); (3) NaHS (310 μg/kg·min); and (4) NaHS (560 μg/kg·min). Fifteen minutes later, a stimulus-response curve was constructed again during the infusion of the above compounds to analyze their effects on the sympathetically-induced tachycardic 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, as previously reported (Centurión et al., 2016).

2.3.2. Protocol 2. Administration of noradrenaline and isoproterenol

The second group of rats (n = 48) was prepared as described above, but the pithing stylet was not replaced by the isolated electrode. After determining baseline values of diastolic blood pressure and heart rate (at least 15 min), the animals were divided into two subgroups (n = 24 each). In the first subgroup, the tachycardic responses were elicited by i.v. bolus injections of noradrenaline. Next, this subgroup was divided into 4 subsets (n = 6 each) that received, respectively, an i.v. continuous infusion of: (1) nothing, to evaluate the reproducibility of the responses; (2) PBS (0.02 ml/min; vehicle of NaHS); (3) NaHS (310 μg/kg·min); and (4) NaHS (560 μg/kg·min). Fifteen minutes later, a second dose-response curve was performed.

Lastly, the second subgroup (n = 24) was divided into 4 subsets that received an i.v. continuous infusion of: (1) nothing (control); (2) PBS (0.02 ml/min; vehicle of NaHS); (3) NaHS (310 μg/kg·min); and (4) NaHS (560 μg/kg·min). Fifteen minutes later i.v. bolus injections of isoproterenol were administered, during the above infusions. As we observed tachyphylaxis after repeating a second dose-response-curve to isoproterenol in the same animal (data not shown), the effect of NaHS or vehicle on isoproterenol-induced tachycardia was determined in independent groups.

When heart rate had returned to baseline values, the next dose was applied; this procedure was performed until the dose-response curve had been completed (60 min).

2.4. Drugs

The drugs used in this work were: gallamine triethiodide, isoproterenol hydrochloride, (±)-noradrenaline bitartrate, sodium chloride, sodium hydrosulfide monohydrate (NaHS), sodium phosphate monobasic monohydrate, (Sigma Chemical Co., St. Louis, MO, U.S.A.),