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Role of central hydrogen sulfide on ventilatory and cardiovascular responses to hypoxia in spontaneous hypertensive rats

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ABSTRACT

Central hydrogen sulfide (H_2S) has been reported to act as a gaseous neuromodulator involved in the ventilatory and cardiovascular control of normotensive rats, whereas no information is available in spontaneously hypertensive rats (SHR). We recorded minute ventilation (V_E), mean arterial pressure (MAP) and heart rate (HR) before and after blocking of enzyme Cystathionine β-synthase (CBS) producing H2 S in neural tissue by microinjection of aminooxyacetate (inhibitor of CBS) into the fourth ventricle of Wistar normotensive rats (WNR) and SHR followed by 30 min of normoxia (21% inspired O_2) or hypoxia (10% inspired O_2) exposure. Microinjection of AOA or saline (1 μL) did not change V_E, MAP and HR during normoxia in both WNR and SHR. In WNR, hypoxia caused an increase in V_E, HR and a decrease in MAP and these responses were unaltered by AOA. In SHR, hypoxia produced a higher increase of V_E, and decrease in MAP and HR when compared to WNR, and these responses were all blunted by AOA. In conclusion, endogenous H_2S plays important modulatory roles on hypoxia-induced ventilatory and cardiovascular responses, inhibiting the cardiovascular and stimulating the respiratory systems in SHR.

1. Introduction

Hydrogen sulfide (H_2S) has been accepted as a new gaseous neuromodulator not only in peripheral tissues but also in the central nervous systems. H_2S is manly produced by two pyridoxal-5′-phosphate-dependent enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (Abe and Kimura, 1996). The CBS isoform is predominantly found in the central nervous system, whereas the CSE isoform predominates in such peripheral tissues as kidney, liver and blood vessels (Yang et al., 2008).

Several of recent studies have investigated the actions of H_2S in a number of conditions. For instance, it has been reported that central H_2S acts as a key mediator of the ventilatory responses to hypercapnia (da Silva et al., 2014) and hypoxia (Donatti et al., 2014; Kwiatkoski et al., 2014). Similarly, central H_2S seems to be involved in blood pressure control in normotensive rats (Dawe et al., 2008; Ufnal et al., 2008; Liu et al., 2011). In the periphery, enhancing of H_2S production has been reported in the carotid body during experimental chemoreflex activation by hypoxia in both normotensive (Peng et al., 2010) and spontaneous hypertensive rats (SHR) (Peng et al., 2014). However, there is no report about the role of central H_2S on respiratory and cardiovascular systems of SHR exposed to hypoxia.

SHR has been accepted as an exceptional experimental model of hypertension, because it shows similar physiological alteration observed in humans, such as sympathetic overactivity and reduced vagal drive (Geraldes et al., 2014; Frohlic and Pfeffer, 1975). Recent studies have indicated that the decreased cardiovascular reflexes sensitivity (Masson et al., 2014; Krieger et al., 2001) and the increased chemoreflex sensitivity (Peng et al., 2014) are involved in the autonomic imbalance and hypertension observed in SHR.

Here, using chronic rats, we tested hypothesis that CBS differentially affects cardio-respiratory parameters in Wistar normotensive rats (WNR) and SHR during eupnea (normoxia) and hypoxia.

2. Material and methods

Experiments were performed on 54 male rats (17–20 weeks old) collectively housed (4 rats per cage) in plastic cages with free access to food and water that were maintained with a 12:12 h light-dark cycle. Twenty six WNR were divided into four groups (number of rats given in parenthesis): normoxia + saline (6), normoxia + AOA (6), hypoxia + saline (6) and hypoxia + AOA (8). Twenty nine SHR were also divided into four groups (number of rats given in parenthesis): normoxia + saline (6), normoxia + AOA (6), hypoxia + saline

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(10) and hypoxia + AOA (7). All experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. The experimental procedures used in this study were approved by the Local Committee of Ethics in the Animal Research of the School of Medicine of Ribeirao Preto, University of São Paulo.

2.1. Drugs

The drug used was the inhibitor of the enzyme CBS [Aminooxyacetate (AOA, 9 nmol in 1 μL), Sigma, St. Louis, MO, USA], which was freshly dissolved in saline on the day of the experiment and the pH verified and adjusted to 7.4 when needed (as previously described Kwiatkoski et al., 2014).

2.2. Surgical procedures

Six days before the experiment, the animals were subjected to surgery for implantation of guide microinjection cannula into 4th ventricle under ketamine-xylazine anesthesia (100 and 10 mg/kg, respectively; 1 mL/kg, i.p.). The animals were fixed on a stereotaxic frame to be implanted with a stainless steel guide cannula (15-mm long, 22-gauge outer diameter) in the fourth ventricle (4 V; for central microinjection) according to the following stereotaxic coordinates (Paxinos and Watson, 2005 (anteroposterior: 11.9 mm caudal from bregma, at the midline, and 7.4 mm ventral to the surface of the skull)). Antibiotics (160,000 U/kg benzylpenicilin, 33.3 mg/kg streptomycin, and 33.3 mg/kg dihydrostreptomycin, i.m.; prophylactically) and analgesic medication (Flunexine; 2.5 mg/kg, s.c.) were provided immediately after the surgery.

Twenty four hours before the experiments, the rats were anesthetized with ketamine-xylazine anesthesia (100 and 10 mg/kg, respectively; 1 mL/kg, i.p.) and polyethylene catheter (filled with pyrogen-free sterile saline) was inserted into the left femoral artery for the direct measurement of arterial pressure. The catheter was tunneled subcutaneously and exteriorized through the back of the neck.

2.3. Measurement of respiratory and cardiovascular parameters

The recordings and analysis of respiratory parameters were performed according to a previous study (da Silva et al., 2014).

The respiratory frequency (F), tidal volume (V_T) and V_E were obtained by whole-body plethysmography in unanesthetized rats (Bartlett and Tenney, 1970) individually placed in the Plexiglas chamber connected to a reference chamber of identical size and construction. The pressure oscillations caused by animal's ventilation were detected by a differential transducer (MLT141 spirometer, Power Lab, AdInstruments, Bella Vista, NSW, Australia). The recordings were analyzed using the software PowerLab (Chart 5; ADInstruments). The volume of calibration was performed during each V_E measurement throughout the course of the experiments by injecting a known volume of air (1 mL) inside the chamber. Body temperature was recorded throughout the experiments at 5-min interval with the temperature datalogger capsule (SubCue, Calgary, AB, Canada) inserted into the peritoneal cavity to calculate tidal volume (V_T). Tidal volume (V_T) was calculated using the following formula (Malan, 1973):

$$V_T = VK \times \frac{P_T}{PK} \times \frac{TA}{TR} \times \frac{PB - PC}{(PB - PC) - \left(TA/Tb\right) \times (PB - PR)}$$

where PT is the pressure deflection associated with each V_T; PK is the pressure deflection associated with injection of the calibration volume (VK); Tb is deep body temperature; TA is the air temperature in the animal chamber; PB is the barometric pressure; PR is the vapour pressure of water at Tb; PC is the vapour pressure of water in the animal chamber; and TR is the room temperature. V_E was calculated as the product of V_T and F. V_E and V_T are presented at the ambient barometric pressure, at deep body temperature, and saturated with water vapor at this temperature (BTPS).

To measure cardiovascular parameters, the arterial catheter was connected to a pressure transducer (MLT0380/D, ADInstruments, Sydney, Australia) and the AP signal was amplified (ML110, ADInstruments, Sydney, Australia), fed to an IBM/PC connected to a Power Lab (ML866, ADInstruments, Sydney, Australia). Mean arterial pressure (MAP) and heart rate (HR) were calculated from the pulsatile arterial pressure (PAP).

2.4. Experimental protocol

Throughout the experiments, including the preceding overnight period, the rats were placed in the experiment room at 25 °C. Each rat was used once only, and received only one microinjection of AOA or vehicle (pyrogen-free sterile saline). A 5-μL Hamilton syringe and a dental injection needle (Missy, 200 μm OD) were used for all the ICV injections. Injection was performed over a period of 30 s, and 30 s was allowed to elapse before the injection needle was removed from the guide cannula to avoid reflux. In all experimental protocols, each animal was individually placed in a Plexiglass chamber, and allowed to freely move while the chamber was ventilated with humidified room air. After acclimatization for 60 min, basal V_E (control) and cardiovascular measurements were taken over a 30-min period. Subsequently, a hypoxic gas mixture (10% O_2, N_2 balance) was flushed into the chamber for 30 min. Control groups were exposed to a normoxic gas mixture (21% O_2, N_2 balance). V_E and cardiovascular parameters were measured over 30 min after the beginning of normoxia/hypoxia exposure. All gas conditions were flushed by a flow meter gas-mixing pump (Columbus Instruments Pegas 4000, OHIO, USA). O_2 gas analyzer (Gas Analyzer, AdInstruments, Sydney, Australia) was used to monitor gas composition inside the chamber in all experimental protocols.

2.4.1. Effect of AOA on the ventilatory and cardiovascular responses to hypoxia

To investigate whether endogenous H_2S plays a role in the pulmonary ventilation and cardiovascular control, unanesthetized, freely moving rats were microinjected with AOA (9 nmol/1 μL) or vehicle (saline 1 μL). Next, the rat was exposed to hypoxia (10% O_2, N_2 balance) or maintained in normoxia (21% O_2, N_2 balance) during 30 min. Ventilatory responses to hypoxia were evaluated at 5, 10, 20 and 30 min, whereas MAP and HR were evaluated each 3 min.

2.5. Statistical analysis

Results are expressed as the mean ± SEM. The basal values [systolic arterial pressure (SAP), diastolic arterial pressure (DAP), MAP, HR, F, V_T and V_E] were evaluated by *t*-test. Changes in response to hypoxia and drugs administration (ΔMAP, ΔHR, ΔF, ΔV_T and ΔV_E) were evaluated using two-way ANOVA for repeated measures followed by the Tukey post-test. The adequacy of the statistical model was determined by drawing probability plots of the residuals using histograms and normal distributions. Differences were considered statistically significant for $P < 0.05$.