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**Involvement of endogenous central hydrogen sulfide (H₂S) in hypoxia-induced
hypothermia in spontaneously hypertensive rats**

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Abstract

Spontaneously hypertensive rats (SHR) display autonomic imbalance and abnormal body temperature (Tb) adjustments. Hydrogen sulfide (H₂S) modulates hypoxia-induced hypothermia but its role in SHR thermoregulation is unknown. We tested the hypothesis that SHR display peculiar thermoregulatory response to hypoxia, and that endogenous H₂S overproduced in the caudal nucleus of the solitary tract (NTS) of SHR modulates this response. SHR and Wistar rats were microinjected into the fourth ventricle with aminooxyacetate (AOA, H₂S-synthesizing enzyme inhibitor) or sodium sulfide (Na₂S, H₂S donor) and exposed to normoxia (21% inspired O₂) or hypoxia (10% inspired O₂, 30 min). Tb was continuously measured, and H₂S production rate was assessed in caudal NTS homogenates. In both groups, AOA, Na₂S or vehicle (saline, 1 µL) did not affect euthermia. Hypoxia caused similar decreases in Tb in both groups. AOA presented a longer latency to potentiate hypoxic hypothermia in SHR. Caudal NTS H₂S production rate was higher in SHR. We suggest that increased bioavailability of H₂S in the caudal NTS of SHR enables the adequate modulation of excitability of peripheral chemoreceptors-activated NTS neurons that ultimately induce suppression of brown adipose tissue thermogenesis thus accounting for the normal hypoxic hypothermia.

Key Words: Hypoxia, Nucleus of the solitary tract, NTS, Anapnoea, SHR, Aminooxyacetate, Cystathionine beta-synthase, Brainstem, Body temperature, Thermoregulation.

1. Introduction

Hypoxia causes a number of compensatory responses in order to maintain oxygen supply to tissues and reduce oxygen consumption when oxygen availability is reduced. During hypoxia, ventilatory and cardiovascular responses are combined with a thermoregulatory adjustment called regulated hypothermia, a controlled decrease in deep body temperature (T_b) which ultimately reduces oxygen consumption thus being vitally beneficial (Wood 1991).

Several neurotransmitters/modulators have been reported to be involved in hypoxic hypothermia, particularly the “third” [along with nitric oxide (NO) and carbon monoxide (CO)] gaseous neuromodulator recently described: hydrogen sulfide (H₂S) (for a review see Branco et al. 2014; da Silva GS et al. 2014; Donatti et al. 2014; Kwiatkoski et al. 2012; Kwiatkoski et al. 2013; Kwiatkoski et al. 2014; Soriano et al. 2012). H₂S is synthesized mainly by two pyridoxal-5'-phosphate-dependent enzymes, cystathionine β -synthase (CBS) and cystathionine γ -lyase (CSE) (Abe and Kimura 1996). CBS is predominantly found in the brain, including the nucleus of the solitary tract (NTS) (Austgen et al. 2011), and CSE is mostly found in the periphery (Yang et al. 2008).

H₂S has been documented to modulate hypothermic response to hypoxia in Wistar rats (Donatti et al. 2014; Kwiatkoski et al. 2012). However, it is not known whether this gasomodulator plays a role in the thermoregulatory system of spontaneously hypertensive rats (SHR) whose sensitivity to hypoxia is increased (Peng et al. 2014) and the autonomic functions and consequently T_b adjustments are abnormal (Campos et al. 2014; Collins et al. 1987; Hajós and Engberg 1986; Morley et al. 1990; O'Donnell and Volicer 1981). Of particular interest to the present report is that endogenous H₂S in the caudal brainstem of SHR regulates ventilatory and

cardiovascular responses to hypoxia (Sabino et al. 2016). Nevertheless, no information is available as to the putative role of H₂S in modulating hypothermic response to hypoxia in SHR, although we know that SHR Tb adjustments are peculiar (Campos et al. 2014; Collins et al. 1987; Hajós and Engberg 1986; Morley et al. 1990; O'Donnell and Volicer 1981) like those seen in hypertensive humans (Kenney 1985; Kenney and Kamon 1984; Kenney et al. 1984).

It has been proposed that SHR carotid bodies (peripheral chemoreceptors) display inherent hypersensitivity to hypoxia (Peng et al. 2014) which would lead one to speculate that SHR exhibit altered hypoxic hypothermia. The afferent fibers of carotid chemoreceptor synapse onto neurons of the caudal NTS which are shown to be activated by hypoxia (Casanova et al. 2013) and modulated by H₂S (Austgen et al. 2011; Malik and Ferguson 2016; Qiao et al. 2011). During hypoxia, peripheral chemoreceptors-activated caudal NTS neurons modulate excitability of rostral raphe pallidus (rRPa) presympathetic neurons which control sympathetic outflow to brown adipose tissue (BAT) and consequently BAT thermogenesis, thus inhibiting rRPa neurons and being permissive for hypoxic hypothermia (Cao et al. 2010; Fyda et al. 1991*b*; Madden and Morrison 2005).

Therefore, given that (i) SHR peripheral chemoreceptors display hypersensitivity to hypoxia (Peng et al. 2014), (ii) SHR Tb adjustments are peculiar (Campos et al. 2014; Collins et al., 1987; Hajós and Engberg 1986; Morley et al. 1990; O'Donnell and Volicer 1981), (iii) CBS is expressed in the NTS (Austgen et al. 2011), and (iv) H₂S modulates (Austgen et al. 2011; Malik and Ferguson 2016; Qiao et al. 2011) excitability of peripheral chemoreceptors-activated caudal NTS neurons which inhibit sympathetic outflow to BAT (Fyda et al. 1991*b*; Madden and Morrison 2005; Cao et al. 2010), we tested the hypothesis that SHR display peculiar

thermoregulatory response to hypoxia, and that this response is modulated by endogenous H₂S whose production is increased in the caudal NTS of SHR.

2. Material and methods

2.1. Animals

Experiments were performed on adult male Wistar rats and SHR (17 weeks old). Experimental protocols were reviewed and approved by the Local Ethical Committees on Animal Experimentation at the University of São Paulo (protocol 064/2012) and were in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals were cared for in accordance with the *Guide to the Care and Use of Experimental Animals* (Vol. 1, 2nd ed., 1993, and Vol. 2, 1984, available from the Canadian Council on Animal Care, Constitution Square, Tower 2, Suite 315, 350 Albert Street, Ottawa, ON K1R 1B1, Canada. The animals were group-housed (four or five animals per cage) and acclimated (12:12-h light-dark cycle) for one week before the experiments. The rats had access water and food *ad libitum*, and were housed in a temperature-controlled chamber at 25 °C (model: ALE 9902001; Alesco Ltda, Monte Mor, SP, Brazil). During the experiments the rats were fully conscious and free to move.

2.2. Drugs

Aminooxyacetate (AOA; 9 nmol/μL, a CBS inhibitor) and sodium sulfide (Na₂S; 4 nmol/μL, a H₂S donor) (Donatti et al., 2014) were purchased from Sigma (S. Louis, MO, USA). These drugs were dissolved in saline just prior to the microinjection procedure (Na₂S) or up to

one week before the experiments, and stored at -20°C (AOA). Drug solutions had pH verified and adjusted to 7.4 when necessary.

2.3. Surgical procedures

Surgery was performed under ketamine-xylazine anesthesia (100 and 10 mg/kg; respectively; 1 ml/kg, i.p.). 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 end of the surgery. The animals were fixed on a stereotaxic frame and had a stainless steel guide cannula (15 mm in length, 22 gauge outer diameter) implanted into the fourth ventricle (for icv microinjection) at the same rostrocaudal level of the NTS, according to the following stereotaxic coordinates [(Paxinos and Watson 2005) (anteroposterior: 11.9 mm, lateral: 0.0 mm, and dorsoventral: 7.4 mm from the bregma)]. The guide cannula was attached to the bone with stainless steel screws and acrylic cement. Tight-fitting stylets were kept inside the cannula to prevent occlusion. The guide cannula was implanted into the fourth ventricle at the same rostrocaudal level of the NTS so as to favor drug diffusion to the NTS (da Silva et al. 2014; Soriano et al. 2012). Accuracy of the cannula placement was checked with a flow of saline into the fourth ventricle by hydrostatic pressure at the moment of its placement, and after the end of the experiments, with a post-mortem microinjection of methylene blue into the fourth ventricle.

Furthermore, a median laparotomy was performed to insert a temperature datalogger capsule (SubCue, Calgary, AB, Canada) into the peritoneal cavity. All animals were kept under deep anesthesia throughout the surgical procedures, receiving a supplementary dose of anesthetic

whenever necessary. Before the experimental procedures, the animals were allowed to recover from surgical interventions for a period of one week.

2.4. Tb recordings

The animals had their Tb continuously recorded at 5-min intervals by the temperature datalogger capsule (SubCue, Calgary, AB, Canada) inserted into the peritoneal cavity.

2.5. Experimental protocols

In all experimental protocols the rats were habituated to the experimental conditions for 60 min in chambers continuously ventilated with humidified room air. At the end of this period, the baseline values for cardiovascular parameters of Wistar rats and SHR as well as their basal Tb were measured (see Supplementary material). After the habituation period, the animals were microinjected into the fourth ventricle and exposed to normoxia (21 % O₂, N₂ balance) or hypoxia (10 % O₂, N₂ balance) for 30 min. Room temperature was maintained at 22°C throughout the experiments. Doses of AOA tested in pilot experiments were based on the literature (da Silva et al. 2014; Kwiatkoski et al. 2012; Kwiatkoski et al. 2013; Kwiatkoski et al. 2014). All gas conditions were flushed by a flow meter gas-mixing pump (Cameron Instruments GF-3/MP; Guelph, ON, Canada). An O₂ gas analyzer (Raytech quadralyser 224A; Middletown, CT, USA) was used to monitor gas composition inside the chamber in all experimental protocols.

2.5.1. Effect of AOA and Na₂S within the fourth ventricle on Tb during normoxia and hypoxia in Wistar rats and SHR

To investigate whether endogenous H₂S plays a role in hypoxia-induced hypothermia, unanesthetized, freely moving rats were microinjected into the fourth ventricle with AOA (9 nmol/μL), Na₂S (4 nmol/μL) or vehicle (saline, 1 μL) and kept under normoxia or exposed to hypoxia. Tb was measured at 5-min intervals for 30 min after the microinjection and exposure to normoxia or hypoxia.

2.5.2. H₂S production rate in homogenates of the caudal NTS of Wistar rats and SHR exposed to normoxia and hypoxia

To test our hypothesis that H₂S production is altered in the caudal NTS of SHR, we measured H₂S production rate in homogenates of the caudal NTS of animals exposed to normoxia or hypoxia. The rats were acclimatized (60 min), kept under normoxia or exposed to hypoxia for 30 min, and immediately after the 30-min exposure they were decapitated and their brains processed. Caudal NTS samples were excised in a cryostat by a punch needle (0.9 mm inner diameter) from a 500-μm thick slice of the brainstem at the level of the caudal NTS.

Caudal NTS samples were homogenized in potassium phosphate buffer (100 mM; pH 7.4) using a microprocessor (VirTis, Gardiner, NY, USA). Each sample (50 % w/v; 100 μl) had L-cysteine (10 mM; 20 μl), pyridoxal 5'-phosphate (2 mM; 20 μl), and PBS (30 μl). The reaction was performed in eppendorf tubes sealed with parafilm, and started by transferring the tubes from ice to bath at 37 °C. After a period of 2 h under incubation, zinc acetate (1 % w/v; 100 μl) was added to trap evolved H₂S followed by trichloroacetic acid (10 % w/v; 100 μl) to precipitate proteins and thus stop the reaction. After centrifugation, *N,N*-dimethyl-*p*-phenylenediamine sulfate (20 mM; 50 μl) in HCl (7.2 M) followed by FeCl₃ (30 mM; 50 μl) in

HCl (1.2 M) was then added to 50 μ l of the supernatant, and optical density was measured at 670 nm. For the calibration curve, measurements were performed based on absorbance using Na₂S solutions (0.1–100 μ g/ml). To measure protein content of the samples, pellets were diluted in 4 ml of sodium hydroxide (0.1 N). The solution was then assayed by using a protein dye reagent (Bio-Rad Laboratories, Hercules, CA, USA; code number: 500-0006). These procedures were previously described elsewhere (da Silva et al. 2014; Francescato et al. 2011; Kwiatkoski et al. 2012; Kwiatkoski et al. 2013; Kwiatkoski et al. 2014; Singh et al. 2009).

2.6. Statistical analysis

The results are expressed as mean $\pm$ S.E.M. Tb values ($^{\circ}$ C) plotted at 5-min intervals are shown as changes (Δ) from basal values of Tb (see Supplementary material). Statistical differences in Tb were assessed by two-way ANOVA for repeated measures followed by Tukey's post hoc test, and in H₂S production rate by two-way ANOVA. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Central actions of AOA and Na₂S on Tb of Wistar rats and SHR exposed to normoxia and hypoxia

Figure 1 shows the time course of Tb after microinjection of vehicle (saline), AOA, or Na₂S in rats exposed to normoxia (panels A and B) or hypoxia (panels C and D).

In Wistar rats, microinjection of saline, AOA, or Na₂S did not alter Tb during normoxia (panel A). Hypoxia (panel C) evoked a decrease in Tb in the saline group compared to baseline [at 5 ($P < 0.017$), 10, 15, 20, 25 and 30 min ($P < 0.001$)] and to the saline group kept under normoxia [at 10 ($P < 0.038$), 15, 20, 25 and 30 min ($P < 0.001$)]. AOA combined with hypoxia caused a higher reduction in Tb compared to the saline group exposed to hypoxia [at 20 ($P < 0.008$), 25 ($P < 0.001$) and 30 ($P = 0.001$) min]. Na₂S administration blunted hypoxia-induced hypothermia [at 15, 20 ($P \leq 0.05$), 25 ($P < 0.043$) and 30 min ($P < 0.024$)].

Regarding SHR, saline, AOA, or Na₂S caused no change in Tb during normoxia either (panel B). Hypoxia (panel D) caused a decrease in Tb in the saline group compared to baseline [at 10 ($P < 0.005$), 15, 20, 25 and 30 min ($P < 0.001$)] and to the saline group kept under normoxia [at 15 ($P < 0.021$), 20 ($P < 0.024$), 25 ($P < 0.002$) and 30 min ($P < 0.001$)]. The drop of Tb during hypoxia (i.e., hypoxia-induced hypothermia) in SHR was similar to that of Wistar rats (both treated with saline). AOA administration to hypoxic SHR potentiated hypoxic hypothermia only at the end of the experimental period [compared to the saline group at 30 min ($P < 0.035$)]. Na₂S administration to SHR had no significant effect on hypoxia-induced hypothermia.

3.2. Production rate of H₂S in homogenates of the caudal NTS of Wistar rats and SHR exposed to normoxia and hypoxia

The production rate of H₂S was measured in homogenates of the caudal NTS of Wistar rats and SHR exposed to normoxia or hypoxia. Hypoxia caused no significant ($P > 0.05$) change in H₂S production rate in the caudal NTS of Wistar rats and SHR. However, of particular interest

to the present study is the fact that SHR presented a significantly ($P < 0.05$) higher production rate of H_2S in the caudal NTS (Fig. 2).

4. Discussion

The present study provides evidence that SHR despite their autonomic dysfunction display normal hypoxic hypothermia. This maintained response seems to, at least in part, result from permanently high levels of H_2S in the caudal NTS regardless of oxygen availability.

SHR exhibit physiopathological features including sympathetic hyperactivity which may account for their abnormal adjustments of Tb (Campos et al. 2014; Collins et al. 1987; Hajós and Engberg 1986; Morley et al. 1990; O'Donnell and Volicer 1981; Okamoto and Aoki 1963; Trippodo and Frohlich 1981). Therefore, the present data shed light on the putative impacts of sympathetic hyperactivity on the adjustments of Tb under conditions of limited oxygen supply which may occur as a consequence of trauma, heart failure, ischemic stroke, etc (for a review see McBryde et al. 2016).

During basal conditions (normoxia) we observed normal heart rate and elevated arterial pressure in SHR (see Supplementary material) as traditionally reported (Campos et al. 2014; Okamoto and Aoki 1963; Totola et al. 2013). The role of endogenous H_2S in modulating hypoxia-induced adjustments in arterial blood pressure and pulmonary ventilation in SHR has been recently assessed by Sabino and colleagues, showing that endogenous H_2S plays important modulatory roles in ventilatory and cardiovascular responses to hypoxia in SHR, inhibiting the cardiovascular responses and stimulating the respiratory system (Sabino et al. 2016). During the control period (normoxia) Tb of SHR was similar to that of Wistar rats, being in agreement

with some previous reports (Campos et al. 2014; Morley et al. 1990) and opposite to other ones (Collins et al. 1987; O'Donnell and Volicser 1981). Interestingly, Morley et al. (1990) have suggested that Tb in SHR is elevated is due to environmental stress during the procedure of Tb measurement and not an internal physiological change.

Methodological Considerations

SHR is widely used as an animal model of sympathetic hyperactivity. Humans show large individual variations related not only to polygenetic disposition but also to environmental factors which are difficult to distinguish. Conversely, SHR have uniform polygenetic disposition and controlled environmental factors leading to uniform changes in each colony. This fact may stand for a key advantage of using SHR.

We exposed SHR to 10% hypoxia for 30 minutes. Even though this condition was enough to induce hypoxic hypothermia, steady state conditions were not reached. However, we have no reasons to believe the results were likely to be different at steady state.

Since in pilot experiments we observed that exposure to hypoxia for more than 30 min was associated with mortality in SHR we had to shorten to 30 min the duration of exposure. Fortunately, exposure to hypoxia for 30 min is enough to induce significant changes in Tb (hypoxia-induced hypothermia).

The role of H₂S in hypoxic hypothermia in the caudal brainstem of Wistar rats and SHR

Our data indicate that endogenous H₂S in the caudal brain stem did not affect euthermia (normothermia) but attenuated hypoxia-induced hypothermia of Wistar rats (Fig. 1), suggesting

that this gasomodulator acts on nucleus or nuclei adjacent to the fourth ventricle attenuating the drop of Tb induced by hypoxia. Conversely, Donatti et al. (2014) have shown that endogenous H₂S in the rostral ventrolateral medulla (RVLM) potentiates the drop of Tb induced by hypoxia. Similarly, Kwiatkoski et al. (2012) have administered AOA and Na₂S within the third ventricle and the preoptic area of the hypothalamus (POA) and observed that endogenous central H₂S potentiates the reduction of Tb induced by hypoxia. Thus, previous studies have indicated that H₂S within the RVLM and POA and given into the third ventricle (which is rostral to the caudal fourth ventricle) plays a cryogenic role. The discrepancy observed when the results of these previous studies were compared to those of the present one may be explained by the different neural substrates involved.

Besides assessing a different brain region, i.e., the caudal brainstem, we have also expanded the scenario by using animals exhibiting intrinsic sympathetic hyperactivity, SHR. Both Wistar rats and SHR showed similar hypoxia-induced hypothermia when the animals were treated with saline (control groups) showing that the regulated decrease of Tb induced by hypoxia is preserved in SHR, despite their autonomic dysfunction. This result do not corroborate our hypothesis that SHR display peculiar hypothermic response to hypoxia due to their carotid body hypersensitivity to hypoxia and sympathetic hyperactivity.

Moreover, we observed that the effect of AOA was still observed in SHR but exhibiting an increased latency, i.e., a significant effect was observed only at the very end of the experimental period, whereas Na₂S failed to produce a significant change (Fig. 1 D). One may speculate that SHR may have a “saturated” H₂S system. Such speculation is supported by the finding that H₂S production is higher in SHR compared to Wistar rats (Fig. 2). Based on these findings, i.e., the increased latency observed in AOA effect in SHR (Fig. 1D) together with the increased

production of H₂S in SHR (Fig. 2), we speculate that a relatively higher amount of the H₂S-synthesizing enzyme is likely to be found in SHR. Therefore, it is reasonable to consider that because of the greater amount of the H₂S-synthesizing enzyme the levels of H₂S were less reduced (or slowly reduced) by AOA at the same dose (9 nmol/μL) given to Wistar rats, explaining the increased latency observed.

H₂S production in the caudal NTS

The drugs (AOA and Na₂S) microinjected into the fourth ventricle, at the same rostrocaudal level of the NTS, diffused through the tissues adjacent to it. The NTS is adjacent to the fourth ventricle walls and has long been documented to be an essential sensory-integrative nucleus particularly involved in the chemoreflex processing (Braga et al., 2007). Besides being modulated by H₂S (Austgen et al. 2011; Malik and Ferguson 2016; Qiao et al. 2011) NTS neurons express the H₂S-synthesizing enzyme CBS (Austgen et al. 2011), exert significant influence on the control of Tb (Cao et al. 2010; Fyda et al. 1991*a,b*; Gordon 2000; Madden and Morrison 2005), and express Fos protein following hypoxia exposure (Casanova et al. 2013). During hypoxia carotid chemoreceptors-activated caudal NTS neurons modulate excitability of rostral raphe pallidus (rRPa) presympathetic neurons controlling the sympathetic outflow to the brown adipose tissue (BAT), thus suppressing BAT thermogenesis and being permissive for hypoxic hypothermia. (Cao et al. 2010; Fyda et al. 1991*b*; Madden and Morrison 2005). Taken together, these reports make plausible to consider that H₂S may have modulated the activity of hypoxia-activated caudal NTS neurons that inhibit rRPa neurons.

Since the caudal NTS is the most important structure of the caudal brain stem adjacent to the walls of the fourth ventricle supposed to be modulated in our hypoxic experimental procedure, we aimed at measuring H₂S production rate in the caudal aspect of this nucleus (Fig. 2). Specifically the caudal NTS plays a key role in mediating the myriad of hypoxia-induced compensatory responses, standing not only as the first termination but also as an integration region of information arising from the peripheral chemoreceptors (carotid bodies) where hypoxia is sensed (Peng et al. 2010; Peng et al. 2014; Prabhakar 2013; Prabhakar and Joyner 2015). Interestingly, it has been reported that SHR carotid bodies (peripheral chemoreceptors) not only display greater H₂S production but also inherent hypersensitivity to hypoxia which is likely to be involved in hypertension in SHR (Peng et al. 2014).

We observed no statistically significant change in H₂S production rate when both Wistar rats and SHR were exposed to hypoxia, indicating that exposure to hypoxia for 30 min did not significantly alter H₂S production in the caudal NTS, although there is a tendency to reduction in Wistar rats (Fig. 2) which is opposite to the augmentation seen in the caudal NTS of Wistar rats exposed to hypercapnia for 30 min (da Silva et al. 2014). Interestingly, H₂S production rate was found to be significantly higher in the caudal NTS of SHR (Fig. 2) whose carotid chemoreceptors also exhibit increased generation of H₂S and are more sensitive to hypoxia (Peng et al. 2014).

Conclusion

Reconciling the available data we speculate that increased H₂S bioavailability in the caudal NTS of SHR modulates excitability of peripheral chemoreceptors-activated caudal NTS neurons

that ultimately induce suppression of brown adipose tissue thermogenesis, thus compensating the putative impact of the peripheral chemoreceptors exaggerated inputs on the activity of caudal NTS neurons and accounting for the normal hypoxic hypothermia.

Draft

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Conflict of interest

The authors declare that there is no conflict of interest.

5. References

- Abe, K., and Kimura, H. 1996. The possible role of hydrogen sulfide as an endogenous neuromodulator. *J. Neurosci.* 16:1066-1071.
- Austgen, J.R., Hermann, G.E., Dantzer, H.A., Rogers, R.C., and Kline, D.D. 2011. Hydrogen sulfide (H₂S) augments synaptic neurotransmission in the nucleus of the solitary tract (NTS). *J. Neurophysiol.* 106:1822-1832.
- Braga, V.A., Soriano, R.N., Bracciulli, A.L., de Paula, P.M., Bonagamba, L.G., Paton, J.F., and Machado, B.H. 2007. Involvement of L-glutamate and ATP in the neurotransmission of the sympathoexcitatory component of the chemoreflex in the commissural nucleus tractus solitarii of awake rats and in the working heart-brainstem preparation. *J. Physiol.* 581(Pt 3):1129-1145.
- Branco, L.G., Soriano, R.N., and Steiner, A.A. 2014. Gaseous mediators in temperature regulation. *Compr Physiol.* 4:1301-1338.
- Campos, H.O., Leite, L.H., Drummond, L.R., Cunha, D.N., Coimbra, C.C., Natali, A.J., and Prímola-Gomes, T.N. 2014. Temperature Control of Hypertensive Rats during Moderate Exercise in Warm Environment. *J. Sports Sci. Med.* 695-701.
- Cao, W.H., Madden, C.J., and Morrison, S.F. 2010. Inhibition of brown adipose tissue thermogenesis by neurons in the ventrolateral medulla and in the nucleus tractus solitarius. *Am. J. Physiol.* 299:R277-R290.
- Casanova, J.P., Contreras, M., Moya, E.A., Torrealba, F., and Iturriaga, R. 2013. Effect of insular cortex inactivation on autonomic and behavioral responses to acute hypoxia in conscious rats. *Behav. Brain. Res.* 253:60-67.

- Collins, M.G., Hunter, W.S., and Blatteis, C.M. 1987. Factors producing elevated core temperature in spontaneously hypertensive rats. *J. Appl. Physiol.* 63:740-745.
- da Silva, G.S., Soriano, R.N., Kwiatkoski, M., Giusti, H., Glass, M.L., and Branco, L.G. 2014. Central hydrogen sulphide mediates ventilatory responses to hypercapnia in adult conscious rats. *Acta Physiol. (Oxf)*. 212:239-247.
- Donatti, A.F., Soriano, R.N., Sabino, J.P., and Branco, L.G. 2014. Involvement of endogenous hydrogen sulfide (H₂S) in the rostral ventrolateral medulla (RVLM) in hypoxia-induced hypothermia. *Brain Res. Bull.* 108:94-99.
- Francescato, H.D., Marin, E.C., Cunha, F.Q., Costa, R.S., Silva, C.G., and Coimbra, T.M. 2011. Role of endogenous hydrogen sulfide on renal damage induced by adriamycin injection. *Arch. Toxicol.* 85:1597-1606.
- Frohlich, E.D., and Pfeffer, M.A. 1975. Adrenergic mechanisms in human hypertension and in spontaneously hypertensive rats. *Clin. Sci. Mol. Med. Suppl.* 2:225s-238s.
- Fyda, D.M., Cooper, K.E., and Veale, W.L. 1991a. Nucleus tractus solitarius lesions alter the metabolic and hyperthermic response to central prostaglandin E₁ in the rat. *J. Physiol.* 442:337-349.
- Fyda, D.M., Cooper, K.E., and Veale, W.L. 1991b. Modulation of brown adipose tissue-mediated thermogenesis by lesions to the nucleus tractus solitarius in the rat. *Brain Res.* 546:203-210.
- Gordon, F.J. 2000. Effect of nucleus tractus solitarius lesions on fever produced by interleukin-1 β . *Auton. Neurosci.* 85:102-110.
- Hajós, M., and Engberg, G. 1986. Emotional hyperthermia in spontaneously hypertensive rats. *Psychopharmacology (Berl)*. 90:170-172.

- Kenney, W.L. 1985. Decreased core-to-skin heat transfer in mild essential hypertensives exercising in the heat. *Clin. Exp. Hypertens.* 7:1165-1172.
- Kenney, W.L., and Kamon, E. 1984. Comparative physiological responses of normotensive and essentially hypertensive men to exercise in the heat. *Eur. J. Appl. Physiol. Occup. Physiol.* 52:196-201.
- Kenney, W.L., Kamon, E., and Buskirk, E.R. 1984. Effect of mild essential hypertension on control of forearm blood flow during exercise in the heat. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol.* 56:930-935.
- Kwiatkoski, M., Soriano, R.N., Francescato, H.D., Batalhao, M.E., Coimbra, T.M., Carnio, E.C., and Branco, L.G. 2012. Hydrogen sulfide as a cryogenic mediator of hypoxia-induced anapyrexia. *Neuroscience*, 201:146-56.
- Kwiatkoski, M., Soriano, R.N., Araujo, R.M., Azevedo, L.U., Batalhao, M.E., Francescato, H.D., Coimbra, T.M., Carnio, E.C., and Branco, L.G. 2013. Hydrogen sulfide inhibits preoptic prostaglandin E2 production during endotoxemia. *Exp. Neurol.* 240:88-95.
- Kwiatkoski, M., Soriano, R.N., da Silva, G.S., Francescato, H.D., Coimbra, T.M., Glass, M.L., Carnio, E.C., and Branco, L.G. 2014. Endogenous preoptic hydrogen sulphide attenuates hypoxia-induced hyperventilation. *Acta Physiol. (Oxf).* 210:913-927.
- Madden, C.J., and Morrison, S.F. 2005. Hypoxic activation of arterial chemoreceptors inhibits sympathetic outflow to brown adipose tissue in rats. *J. Physiol.* 566(Pt 2):559-573.
- Malik, R., and Ferguson, A.V. 2016. Hydrogen sulfide depolarizes neurons in the nucleus of the solitary tract of the rat. *Brain Res.* 1633:1-9.
- McBryde, F.D., Malpas, S.C., and Paton, J.F. 2016. Intra-cranial mechanisms for preserving brain blood flow in health and disease. *Acta Physiol. (Oxf).* doi: 10.1111/apha.12706 (in press).

- Morley, R.M., Conn, C.A., Kluger, M.J., and Vander, A.J. 1990. Temperature regulation in biotelemetered spontaneously hypertensive rats. *Am. J. Physiol.* 258:R1064-1069.
- O'Donnell, A., and Volicer, L. 1981. Thermoregulation in spontaneously hypertensive rats: effects of antihypertensive treatments. *Clin. Exp. Hypertens.* 3:555-67.
- Okamoto, K., and Aoki, K. 1963. Development of a strain of spontaneously hypertensive rats. *Jpn. Circ. J.* 27:282-293.
- Paxinos, G., and Watson, C. 2005. *The rat brain in stereotaxic coordinates*, 5th ed. New York, Academic Press.
- Peng, Y.J., Nanduri, J., Raghuraman, G., Souvannakitti, D., Gadalla, M.M., Kumar, G.K., Snyder, S.H., and Prabhakar, N.R. 2010. H₂S mediates O₂ sensing in the carotid body. *Proc. Natl. Acad. Sci. U.S.A.* 107:10719-24.
- Peng, Y.J., Makarenko, V.V., Nanduri, J., Vasavda, C., Raghuraman, G., Yuan, G., Gadalla, M.M., Kumar, G.K., Snyder, S.H., and Prabhakar, N.R. 2014. Inherent variations in CO-H₂S-mediated carotid body O₂ sensing mediate hypertension and pulmonary edema. *Proc. Natl. Acad. Sci. U.S.A.* 111:1174-9.
- Prabhakar, N.R. 2013. Sensing hypoxia: physiology, genetics and epigenetics. *J. Physiol.* 591(9):2245-2257.
- Prabhakhar, N.R., and Joyner, M.J. 2015. Tasting arterial blood: what do the carotid chemoreceptors sense? *Front. Physiol.* 5:524.
- Qiao, W., Yang, L., Li, X.Y., Cao, N., Wang, W.Z., Chai, C., and Lu, Y. 2011. The cardiovascular inhibition functions of hydrogen sulfide within the nucleus tractus solitarii are mediated by the activation of KATP channels and glutamate receptors mechanisms. *Pharmacazie.* 66(4):287-292.

Sabino, J.P., Traslaviña, G.A., and Branco, L.G. 2016. Role of central hydrogen sulfide on ventilatory and cardiovascular responses to hypoxia in spontaneous hypertensive rats. *Respir. Physiol. Neurobiol.* 231:21-27.

Singh, S., Padovani, D., Leslie, R.A., Chiku, T., and Banerjee, R. 2009. Relative contributions of cystathionine beta-synthase and gamma-cystathionase to H₂S biogenesis via alternative trans-sulfuration reactions. *J. Biol. Chem.* 284:22457-22466.

Soriano, R.N., Kwiatkoski, M., Batalhao, M.E., Branco, L.G., and Carnio, E.C. 2012. Interaction between the carbon monoxide and nitric oxide pathways in the locus coeruleus during fever. *Neuroscience*, 206:69-80.

Totola, L.T., Alves, T.B., Takakura, A.C., Ferreira-Neto, H.C., Antunes, V.R., Menani, J.V., Colombari, E., and Moreira, T.S. 2013. Commissural nucleus of the solitary tract regulates the antihypertensive effects elicited by moxonidine. *Neuroscience*, 250:80-91.

Trippodo, N.C., and Frohlich, E.D. 1981. Similarities of genetic (spontaneous) hypertension. Man and rat. *Circ. Res.* 3:309-319.

Wood, S.C. 1991. Interactions between hypoxia and hypothermia. *Annu. Rev. Physiol.* 53:71-85.

Yang, G., Wu, L., Jiang, B., Yang, W., Qi, J., Cao, K., Meng, Q., Mustafa, A.K., Mu, W., Zhang, S., Snyder, S.H., and Wang, R. 2008. H₂S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science*, 322:587-590.

Figure captions

Fig. 1: Changes in deep body temperature (T_b) after microinjection of saline (1 µl), AOA (9 nmol/µL) or Na₂S (4 nmol/µL) in Wistar rats and SHR followed by exposure to normoxia (top panels) or hypoxia (bottom panels). Number of animals per group is shown in parenthesis. **P* < 0.05 compared to saline Wistar group.

Fig. 2: Production rate of H₂S in homogenates of the caudal NTS of Wistar rats and SHR exposed to normoxia or hypoxia. Number of animals per group is shown in parenthesis. **P* < 0.05 compared to Wistar group.

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297x209mm (300 x 300 DPI)

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