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Research report

Cryogenic role of central endogenous hydrogen sulfide in the rat model of endotoxic shock

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ABSTRACT

Thermoregulatory responses to lipopolysaccharide (LPS) are affected by modulators that increase (pyretic) or decrease (cryogenic) body temperature (Tb). We tested the hypothesis that central hydrogen sulfide (H_2S) acts as a thermoregulatory modulator and that H_2S production in the anteroventral preoptic region of the hypothalamus (AVPO) is increased during hypothermia and decreased during fever induced by bacterial lipopolysaccharide (LPS, 2.5 mg/kg i.p.) in rats kept at an ambient temperature of 25 °C. Deep Tb was recorded before and after pharmacological inhibition of the enzyme cystathionine β-synthase (CBS – responsible for H_2S endogenous production in the brain) combined or not with LPS administration. To further investigate the mechanisms responsible for these thermoregulatory adjustments, we also measured prostaglandin D_2 (PGD_2) production in the AVPO. LPS caused typical hypothermia followed by fever. Levels of AVPO H_2S were significantly increased during hypothermia when compared to both euthermic and febrile rats. Intracerebroventricular (icv) microinjection of aminooxyacetate (AOA, a CBS inhibitor; 100 pmol) neither affected Tb nor basal PGD_2 production during euthermia. In LPS-treated rats, AOA caused increased Tb values during hypothermia, along with enhanced PGD_2 production. We conclude that the gaseous messenger H_2S modulates hypothermia during endotoxic shock, acting as a cryogenic molecule.

1. Introduction

Sepsis is the systemic response to serious infection that manifests as a continuum of illness from mild vital sign abnormalities to multiple organ dysfunctions (Bentzer et al., 2015; Laszlo et al., 2015). The growing incidence of sepsis is associated with mortality rates of 20–50% in the USA (Gaieski et al., 2013) and despite great advances in our understanding of its pathophysiology and the use of supportive treatments in intensive care units (for instance: vasopressor agents, antibiotic treatment and fluid reposition) the morbidity and mortality rates remain inaptly high worldwide (Alberti et al., 2005; Angus and Wax, 2001). During sepsis important body temperature (Tb) changes are observed: ranging from hypothermia (an energy-conservation strategy that reduces oxygen demand) to fever (an energy-consuming strategy that slow microorganism growth and enhance host immune responses) (Krall et al., 2010). These changes are thought to be triggered by signals in the periphery and to be modulated by the brain, where the anteroventral preoptic region of the hypothalamus (AVPO) plays a major thermoregulatory role. Not only the gaseous molecule nitric oxide (Steiner et al., 2002a) but also hydrogen sulfide (H_2S) have been reported to be endogenously produced gaseous modulators (Kimura, 2015) that affects Tb regulation by acting in the AVPO, during hypoxia-induced hypothermia (Kwiatkoski et al., 2012) and fever induced by low doses of LPS (Kwiatkoski et al., 2013). However, nothing is known about the H_2S role during thermoregulatory responses to sepsis-like conditions, where Tb changes include hypothermia followed by fever (Romanovsky et al., 1996).

In 1979, PGD_2 was reported to be a major PG in the brain (Shimizu et al., 1979). Interestingly Ueno (Ueno et al., 1982) showed that PGD_2 participates in the LPS-induced hypothermic phase. Moreover, in agreement with the notion that PGD_2 plays a cryogenic role, Krall showed that PGD_2 plays a major role in the drop in Tb induced by food deprivation (Krall et al., 2010).

Considering that in the AVPO, H_2S has been reported to induce thermoregulatory adjustments not only during hypothermia at

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least that induced by hypoxia (Kwiatkoski et al., 2012) but also fever induced by low doses of LPS (Kwiatkoski et al., 2013), the goal of this study was to investigate whether H_2S modulates changes in Tb (that includes both hypothermia and fever) observed during sepsis-like conditions (induced with high dose of LPS), using a pharmacological inhibitor of cystathionine β-synthase (CBS – enzyme responsible for H_2S endogenous production in the CNS). To further investigate the mechanisms responsible for these responses we measured PGD_2 (known to act as a key cryogenic mediator) production in the AVPO and plasma during hypothermia and fever.

2. Results

2.1. Effect of administration of AOA on Tb during euthermia

Before investigating the effect of AOA on the thermoregulatory responses to endotoxemia, we evaluated whether the CBS inhibitor would affect Tb control during euthermia. As expected, icv microinjection of AOA (100 pmol) did not cause any significant effect on Tb when compared to the saline-treated group ($P > 0.05$; Fig. 1).

2.2. Effect of AOA on LPS-induced hypothermia and fever

The basal Tb values (euthermia) of the saline and AOA groups before microinjections and injection of LPS are shown (thermal index, Saline: 138.4 ± 9.162 °C × min, n=8; AOA: 139.1 ± 11.54 °C × min, n=8; Fig. 2B). LPS administration (2.5 mg/kg) to rats exposed to 25 °C caused the typical decrease of Tb (hypothermia) in the first 90 min followed by an increase in Tb until the end of the experimental period. When LPS treatment was combined with icv administration of AOA we observed an attenuated drop in Tb during the hypothermic phase ($P=0.0192$; 95% CI: −64.56 to −1.892; AOA: 132.8 ± 11.61 °C × min, n=8; Saline: 90.88 ± 10.79 °C × min, n=8; Fig. 2B) and an increased fever ($P > 0.05$; AOA: 178.8 ± 14.53 °C × min, n=8; Saline: 151.8 ± 12.38 °C × min, n=8; Fig. 2B).

2.3. AVPO production rate of H_2S

LPS injection induced an increased H_2S production rate in the AVPO of hypothermic rats when compared to euthermia and fever groups ($P=0.025$; 95% CI: −0.855 to −0.054; Hypothermia: 1.269 ± 0.172 µg/mg protein/h, n=6; Euthermia: 0.814 ± 0.052 µg/mg protein/h, n=6; Fever: 0.919 ± 0.059 µg/mg protein/h, n=6; Fig. 3A).

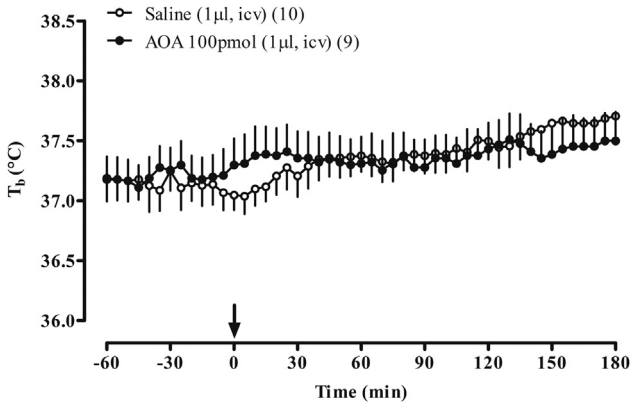

Fig. 1. Effect of AOA on Tb during euthermia. Time courses showing the effect of icv microinjection of AOA (100 pmol/1 µl) or vehicle (saline/1 µl) on T_b of euthermic rats. Arrow indicates the moment of the icv microinjection immediately followed by ip injection. Values are means ± SEM. Number of animals in each group is shown in parenthesis.

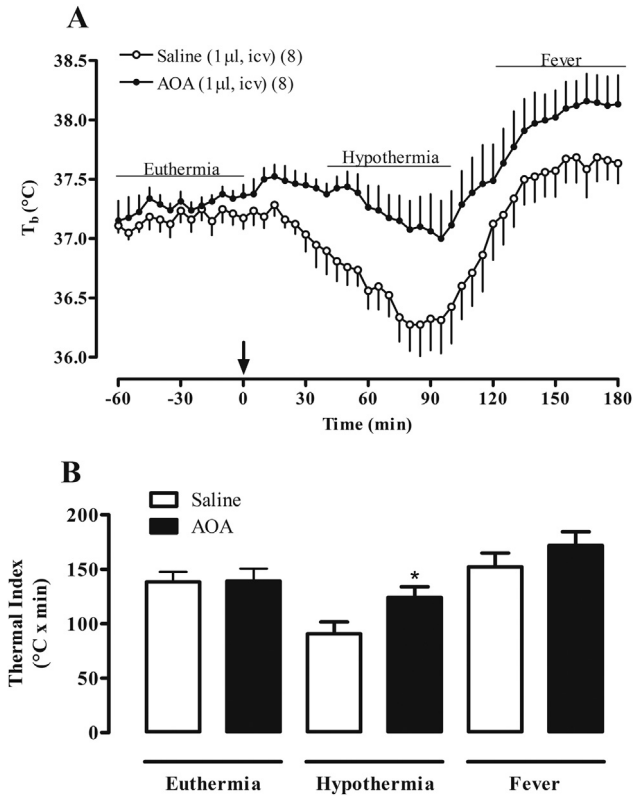

Fig. 2. Effect of AOA on thermoregulation during the LPS shock. A: Time courses showing the effect of icv microinjection of AOA (100 pmol/1 µl) or vehicle (saline/1 µl) on LPS-induced hypothermia and fever (2.5 mg/kg, ip). Arrow indicates the moment of the ip injection and icv microinjection. B: Thermal indexes (area under curve indicated in panel A by horizontal lines) calculated from euthermic, hypothermic and febrile rats. Values are means ± SEM. Number of animals in each group is shown in parenthesis. *$P < 0.05$ vs. control group.

2.4. AVPO levels of PGD_2

In euthermic rats, icv microinjection of AOA did not affect PGD_2 production ($P > 0.05$; Saline: 0.2855 ± 0.05033 pg/mg protein, n=6; AOA: 0.268 ± 0.046 pg/mg protein, n=6; Fig. 4). Surprisingly, AOA evoked increased levels of AVPO PGD_2 in hypothermic rats ($P=0.01$; 0.435 ± 0.044 pg/mg protein, n=6) when compared to febrile ones (0.423 ± 0.044 pg/mg protein, n=6) and the icv saline-treated groups (0.244 ± 0.022 pg/mg protein, n=6 and 0.321 ± 0.013 pg/mg protein, n=6; Fig. 4).

2.5. Plasma levels of PGD_2

We observed increased plasma PGD_2 levels in hypothermic (14.233 ± 1.962 pg/ml, n=6) and febrile rats (12.700 ± 2.155 pg/ml, n=6) in comparison to euthermic rats (7.683 ± 1.480 pg/ml, n=6). The icv microinjection of AOA (100 pmol) did not cause any significant effect on plasma PGD_2 production either during euthermia (8.43333 ± 1.93918 pg/ml, n=6), hypothermia (11.667 ± 1.686 pg/ml, n=6) or fever (10.233 ± 1.604 pg/ml, n=6) (Fig. 5).

3. Discussion

The present study provides robust evidence that H_2S is a key modulator of the thermoregulatory responses to endotoxic shock.