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HYPOXIA-INDUCED HYPOTHERMIA MEDIATED BY GABA IN THE ROSTRAL PARAPYRAMIDAL AREA OF THE MEDULLA OBLONGATA

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Abstract—Hypoxia evokes a regulated decrease in the body core temperature (T_c) in a variety of animals. The neuronal mechanisms of this response include, at least in part, glutamatergic activation in the lateral preoptic area (LPO) of the hypothalamus. As the sympathetic premotor neurons in the medulla oblongata constitute a cardinal relay station in the descending neuronal pathway from the hypothalamus for thermoregulation, their inhibition can also be critically involved in the mechanisms of the hypoxia-induced hypothermia. Here, I examined the hypothesis that hypoxia-induced hypothermia is mediated by glutamate-responsive neurons in the LPO that activate GABAergic transmission in the rostral raphe pallidus (rRPa) and neighboring parapyramidal region (PPy) of the medulla oblongata in urethane–chloralose-anesthetized, neuromuscularly blocked, artificially ventilated rats. Unilateral microinjection of GABA (15 nmol) into the rRPa and PPy regions elicited a prompt increase in tail skin temperature (T_s) and decreases in T_c, oxygen consumption rate (VO_2), and heart rate. Next, when the $GABA_A$ receptor blocker bicuculline methiodide (bicuculline methiodide (BMI), 10 pmol) alone was microinjected into the rRPa, it elicited unexpected contradictory responses: simultaneous increases in T_s, VO_2 and heart rate and a decrease in T_c. Then, when BMI was microinjected bilaterally into the PPy, no direct effect on T_s was seen; and thermogenic and tachycardic responses were slight. However, pretreatment of the PPy with BMI, but not vehicle saline, greatly attenuated the hypothermic responses evoked by hypoxic (10%O_2–90%N_2, 5 min) ventilation or bilateral microinjections of glutamate (5 nmol, each side) into the LPO. The results suggest that hypoxia-induced hypothermia was mediated, at least in part, by the activation of $GABA_A$ receptors in the PPy. © **2014 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: anapyrexia, rostral ventromedial medulla, thermoregulation, preoptic area.

INTRODUCTION

In a hypoxic environment, a wide variety of animals exhibit a decrease in their body core temperature (T_c) (Wood, 1991; Steiner and Branco, 2002). This response, referred to as anapyrexia, is achieved by a coordinated increase in heat loss and decrease in heat production. A lower T_c is beneficial for animals in hypoxic situations (Artru and Michenfelder, 1981; Wood and Stabenau, 1998), because it causes a leftward shift of the O_2-hemoglobin dissociation curve, resulting in an increase in the affinity of hemoglobin for O_2 in the lung, and because it reduces the O_2 demand of the tissue per se. Hypoxia-induced hypothermia is regulated centrally (Wood, 1991; Steiner and Branco, 2002; Steiner et al., 2002; Madden and Morrison, 2005; Osaka, 2011, 2012b), though a peripheral action has also been suggested (Johnson et al., 2007).

Thermoregulatory heat loss is largely dependent on cutaneous vasomotion and blood flow in glabrous skin, such as the tail of rats and ear pinna of rabbits. An increase in blood flow in ear pinna of rabbits (Blessing et al., 1999) and that in the rat tail (Blessing and Nalivaiko, 2001) are induced by inhibition of the rostral raphe pallidus (rRPa) and neighboring parapyramidal region (PPy) in the rostral ventromedial medulla oblongata by microinjection of GABA or the $GABA_A$ agonist muscimol. Consistently, the microinjection of muscimol into the rRPa and PPy regions also inhibits cutaneous sympathetic nerve activity in anesthetized rats and rabbits (Ootsuka et al., 2004; Ootsuka and McAllen, 2005; Rathner et al., 2008) and elicits cutaneous vasodilation and hypothermia in conscious rats (Zaretsky et al., 2003; Cerri et al., 2010, 2013); whereas chemical stimulation of the rRPa with microinjected glutamate increases tail sympathetic nerve activity in anesthetized rats (Rathner and McAllen, 1999; Ootsuka and McAllen, 2005). Indeed, the rRPa and PPy regions constitute a cardinal relay station in the descending neural pathway from the hypothalamus for the control of thermoregulatory cutaneous vasomotion and heat dissipation from the skin (Nalivaiko and Blessing, 2001; Korsak and Gilbey, 2004; Rathner et al., 2008; Tanaka et al., 2002, 2011, 2013). Therefore, it is possible that GABAergic inhibition of the sympathetic premotor neurons in the rRPa and PPy regions is the critical step in the medullary mechanisms of hypoxia-induced heat-loss responses, though there is no proof for this hypothesis. Similarly, the rRPa contains sympathetic premotor neurons that regulate brown

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Abbreviations: BMI, bicuculline methiodide; GABA, gamma-aminobutyric acid; LPO, lateral preoptic area; PPy, parapyramidal region; rRPa, rostral raphe pallidus; T_c, body core temperature; T_s, skin temperature of the tail; VO_2, rate of whole-body O_2 consumption; VCO_2, rate of whole-body CO_2 production.

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adipose tissue (BAT) (Morrison, 1999; Morrison et al., 2012; Nakamura et al., 2002, 2004), the sole tissue in mammals specialized for heat production. Disinhibition of neurons in the rRPa by microinjection of the $GABA_A$ antagonist bicuculline elicits BAT thermogenesis (Morrison et al., 1999), and this response is reversed by hypoxic stimulation (Madden and Morrison, 2005). However, the neuronal circuit responsible for this interaction also has not been elucidated.

Chemical stimulation of the lateral preoptic area (LPO) with microinjected glutamate also elicits heat loss and hypothermic responses (Osaka, 2012a); whereas pretreatment of this area with a microinjected nonselective glutamate receptor antagonist, kynurenic acid, greatly attenuates hypoxia-induced hypothermia (Osaka, 2012b). Conversely, inhibition of the LPO with microinjected GABA elicits thermogenesis (Osaka, 2012a) and activation of the tail sympathetic fibers (Tanaka et al., 2009). Therefore, it is likely that hypoxia-induced hypothermia is mediated, at least in part, by activation of the glutamatergic transmission in the LPO. Because a number of neurons in the LPO project directly to the rostral medullary raphe region (Yoshida et al., 2009), I examined the hypothesis that the glutamate-responsive neurons in the LPO activate the GABAergic transmission in the rRPa and PPy regions to mediate hypoxia-induced hypothermia. For this purpose, I first investigated the effective locus to evoke the tail skin vasodilatory and hypothermic effects of GABA in the rRPa and PPy regions. Then, I examined the effects of pretreatment of this GABA-responsive locus with the $GABA_A$ antagonist bicuculline methiodide (BMI) on the hypothermic responses induced by the hypoxic ventilation or the microinjection of glutamate into the LPO.

EXPERIMENTAL PROCEDURES

Adult male Wistar rats, weighing 350–420 g, were maintained at the ambient temperature of 23 ± 1 °C for > 1 week before the experiments. The experimental procedures, which included minimizing the number of animals used and their suffering, conformed to the NIH guidelines on the ethical use of animals and were approved by the Ethics Committee of the National Institute of Health and Nutrition, Japan.

After induction of anesthesia with 2–3% isoflurane in air and cannulation of a femoral vein and the trachea, the rats were kept anesthetized by i.v.-injected urethane (550 mg kg^{-1}) and α-chloralose (55 mg kg^{-1}). The animals were placed in a stereotaxic apparatus with their head fixed according to the coordinate system of Paxinos and Watson (2007). Their T_c was maintained at ~37 °C with a heating pad prior to microinjections or hypoxia, whereas their tail was placed outside of the pad so as not to induce vasodilation of the tail by warming. The animals were

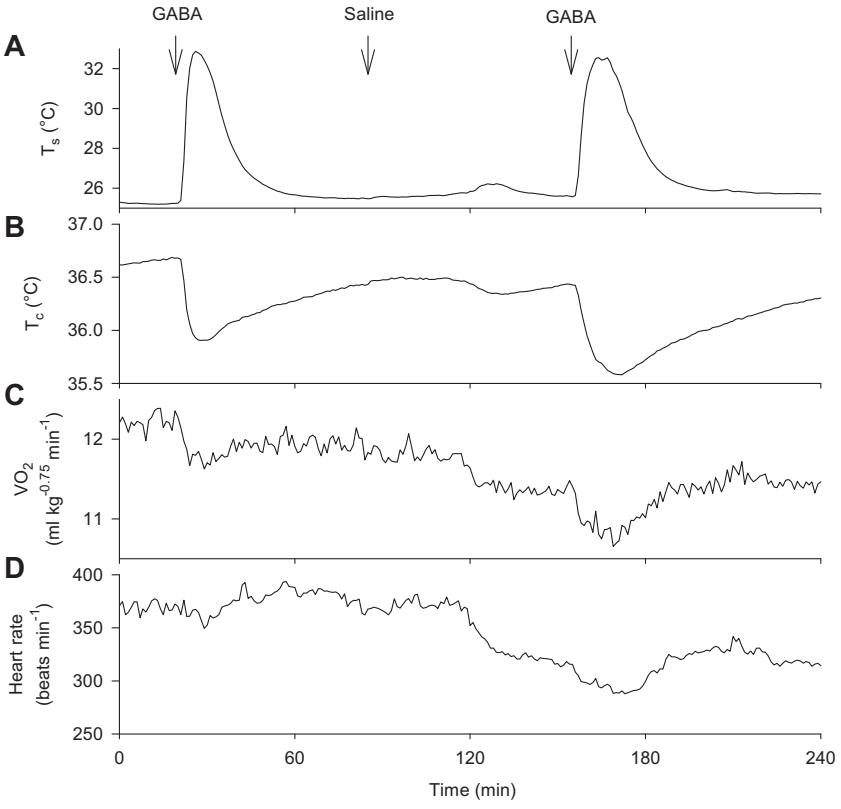

Fig. 1. Representative records demonstrating effects of unilateral microinjection of GABA (15 nmol/50 nl) or physiological saline into the same site in the border of the rRPa and PPy on T_s (A), T_c (B), VO_2 (C), and heart rate (D) in a rat. In spite of a small spontaneous increase in T_s and simultaneous decreases in T_c, VO_2, and heart rate at around 120 min, GABA microinjected a second time thereafter reproducibly increased T_s and decreased T_c, VO_2, and heart rate.