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Research report

Changes of body temperature and thermoregulatory responses of freely moving rats during GABAergic pharmacological stimulation to the preoptic area and anterior hypothalamus in several ambient temperatures

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Abstract

Action of γ-aminobutyric acid (GABA) in the preoptic area and anterior hypothalamus (PO/AH) has been implicated to regulate body temperature (T_b). However, its precise role in thermoregulation remains unclear. Moreover, little is known about its release pattern in the PO/AH during active thermoregulation. Using microdialysis and telemetry techniques, we measured several parameters related to thermoregulation of freely moving rats during pharmacological stimulation of GABA in normal (23 °C), cold (5 °C), and hot (35 °C) ambient temperatures. We also measured extracellular GABA levels in the PO/AH during cold (5 °C) and heat (35 °C) exposure combined with microdialysis and high performance liquid chromatography (HPLC). Perfusion of $GABA_A$ agonist muscimol into the PO/AH increased T_b, which is associated with increased heart rate (HR), as an index of heat production in all ambient temperatures. Although tail skin temperature (T_{tail}) as an index of heat loss increased only under normal ambient temperatures, its response was relatively delayed in comparison with HR and T_b, suggesting that the increase in T_{tail} was a secondary response to increased HR and T_b. Locomotor activity also increased in all ambient temperatures, but its response was not extraordinary. Interestingly, thermoregulatory responses were different after perfusion of $GABA_A$ antagonist bicuculline at each ambient temperature. In normal ambient temperature conditions, perfusion of bicuculline had no effect on any parameter. However, under cold ambient temperature, the procedure induced significant hypothermia concomitant with a decrease in HR in spite of hyperactivity and increase of T_{tail}. It induced hyperthermia with the increase of HR but no additional change of T_{tail} in hot ambient temperature conditions. Furthermore, the extracellular GABA level increased significantly during cold exposure. Its release was lower during heat exposure than in a normal environment. These results indicate that GABA in the PO/AH is an important neurotransmitter for disinhibition of heat production and inhibition of heat loss under cold ambient temperature. It is a neurotransmitter for inhibition of heat production under hot ambient temperature.

Theme: Endocrine and autonomic regulation
Topic: Osmotic and thermal regulation

Keywords: γ-Aminobutyric acid; Thermoregulation; Preoptic area and anterior hypothalamus; Microdialysis; Telemetry; Freely moving rats

1. Introduction

The preoptic area and anterior hypothalamus (PO/AH) is considered to be the primary locus for integration of thermal signals originating from different parts of the body. It

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coordinates body temperature regulation (T_b) [4,14,18,20]. Many studies have shown that thermal stimulation of the PO/AH either facilitates or inhibits thermoregulatory responses [6,14,19,20]. Furthermore, electric and chemical stimulation of the PO/AH also changes T_b [8,29]. Recent studies show interesting results regarding the regulation of heat production in the PO/AH. Activation of warm-sensitive neurons in the PO/AH by warming or injection of the excitatory amino acid D,L-homocysteric acid to the PO/AH suppresses shivering [29] and non-shivering [8] heat production responses. We also recently reported that, in freely moving rats, inhibition of neurons in the PO/AH by perfusion of tetrodotoxin induced an increase in T_b with increased heat production response [15]. These results suggest that the functional role of the PO/AH in heat production system is inhibitory control against other loci that regulate heat production responses. However, neurotransmitters in the PO/AH that mediate this inhibitory control remain unknown despite abundant pharmacological evidence that implicates it in regulation of a variety of monoamines, amino acids, and peptides [9,10].

In that regard, we specifically examined γ-aminobutyric acid (GABA) because it is a predominant inhibitory neurotransmitter in the hypothalamus [3,11]. Moreover, it is more abundant, especially in the PO/AH, than in other brain regions [1,28]. Concerning the relationship between GABA and thermoregulation, numerous reports have presented interesting data. For example, GABA in the PO/AH is reportedly involved in both heat loss and heat production responses [1,2]. It is also reported that GABA affects temperature-sensitive neurons in the PO/AH in experiments using GABA agonist or antagonist in brain tissue slices [27] and anesthetized rats [17]. In addition, Osborne et al. [23] used microdialysis technique and reported that perfusion of muscimol, $GABA_A$ agonist, into the PO/AH increased T_b in freely moving rats. That study concluded that hyperthermia is independent of fever or hyperactivity, but the underlying mechanism of such an increase in T_b was not clarified (increase of heat production and/or decrease of heat loss) because other parameters related to measurement of T_b regulation were not recorded simultaneously. Nevertheless, another study under anesthetized conditions found that hyperthermia accompanied heat production response (increase in heart rate) but not heat loss response (no change in cutaneous blood flow) [22]. Furthermore, Osaka [21] also reported, under anesthetized conditions, that GABA-induced hyperthermia in the PO/AH resulted from activation of heat production response (increase in O_2 consumption) but not heat loss response (no change tail skin temperature). These results indicated that GABA in the PO/AH is involved mainly in thermogenesis regulation, especially in disinhibition of heat production. However, no studies have examined the detailed influence of GABA on thermoregulation without anesthetization.

This study examined thermoregulatory responses after GABA stimulus of conscious rats in various ambient temperatures (5, 23, and 35 °C) to confirm the role of GABA in the PO/AH. Specifically, we simultaneously measured T_b, tail skin temperature (T_{tail}) as an index of heat loss [7,12], heart rate (HR) as an index of heat production [6,19], and locomotor activity (Act) in freely moving rats. Furthermore, little is known about the pattern of GABA release in the PO/AH during active thermoregulation. For that reason, we also measured its level during cold (5 °C) and heat (35 °C) exposure besides the experiment on the pharmacological stimulation. Subsequently, we combined those results with those of microdialysis and high performance liquid chromatography (HPLC).

2. Materials and methods

2.1. Animals

Male Wistar rats (250–350 g body weight) were housed separately in plastic cages under controlled conditions of ambient temperature (23 °C), relative humidity (50%), and a 12/12 h light/dark cycle (lights on at 06:00 h). Rats had free access to food and water except during experiments. All experiments were carried out according to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan.

2.2. Thermoregulatory parameters measurements

Pentobarbital (50 mg/kg, i.p.) anesthesia was used to allow implantation of a telemetry device (TA10ETA-F20; Data Sciences International, USA) in the peritoneal cavity, which allowed continuous monitoring of T_b, HR, and Act in freely moving rats. We measured T_{tail} on the dorsal surface of the skin about 10 mm from the tail base using an alumel–chromel thermocouple wire. The thermocouple wire was covered with a plastic tube and a metal spring. We also used the 'Nejiren' (RC-2000; Osaka Microsystems, Japan) to prevent tangling of the thermocouple and microdialysis tubes. Using a temperature-controlled chamber, the ambient temperature was set at 23 °C (normal environment), 35 °C (hot environment), and 5 °C (cold environment) for 3 h to elicit changes in the rats' thermal balance [15,16].

2.3. Microdialysis

At least 7 days were allowed between insertion of telemetry sensors and microdialysis preparation. The rats were anesthetized with pentobarbital (50 mg/kg) 2–3 days before the experiments. At that time, the microdialysis probe (0.24 mm external diameter, 2.0-mm-long dialyzing membrane, and molecular weight cutoff value of 6000 cuprophane membrane, CUP 11; CMA Microdialysis AB, Sweden) was