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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

placed stereotaxically in the left lateral PO/AH (coordinates from bregma: AP −0.4 mm; L +0.3 mm; D −8.3 mm from dura) [24]. Microdialysis perfusion (1 μl/min) commenced at 08:00 h over 10 h. Before pharmacological stimulation, we spent sufficient time (at least 4 h) until thermoregulatory parameters stabilized. Muscimol (500 μM) and bicuculline (50 μM) were perfused for 60 min into the PO/AH. Ringer's solution (in mM, 147 NaCl, 4 KCl, 2.3 $CaCl_2$, pH 6.0) [16,28] was perfused continuously, except during dialysis of the above substances. Those aforementioned agents were perfused separately during the last hour of each exposure in heat and cold exposure experiments. Microdialysis sampling was done besides the experiment on the pharmacological stimulation and started after 2 h of Ringer perfusion. After 3 h of baseline collections, rats randomly received cold or heat thermal stimulation. Microdialysate was collected into microvials containing 5 μl of 0.02 M acetic acid to minimize auto-oxidation. The collection procedure was repeated using a refrigerated fraction collector (CMA 170; CMA Microdialysis AB) for analysis of extracellular GABA levels in the PO/AH.

2.4. HPLC

The microdialysate was analyzed using HPLC. Identification of unknown peaks in samples was accomplished by matching the retention time of peak with that of an authentic standard (Chromograph Report Software; Bioanalytical Systems, Inc. (BAS), USA). For analysis of GABA, we used *o*-phthalaldehyde (OPA) derivatives with fluorometric detection (CMA 280 detector; CMA Microdialysis AB) and a pump (PM-70; BAS, Japan). We used 5-μm monomeric C-18 columns (1.0 mm i.d. × 10 cm, BAS). The mobile phase consisted of 0.1 M acetate buffer and 20% acetonitrile at pH 5.4. The flow rate was 60 μl/min. Samples of 20 μl were mixed automatically with 5 μl of OPA reagent (CMA 200; CMA Microdialysis AB); they were then reacted for 90 s at 10 °C. The reaction product was injected automatically into the HPLC by a robot (CMA 200, CMA Microdialysis AB). An aliquot of 99% acetonitrile was injected automatically 14 min after the run to flush out substances that remained in the column. Details of temperature measurements, microdialysis, and HPLC methods have been described elsewhere [15,16,28].

2.5. Drugs

Muscimol ($GABA_A$ agonist) and bicuculline ($GABA_A$ antagonist) were obtained from Sigma-Aldrich (St. Louis, MO). All drugs were dissolved in Ringer's solution.

2.6. Histological examination

At the end of each experiment, rats were sacrificed using an overdose of pentobarbital (120 mg/kg, i.p.). The microdialysis probe placement was verified, using a photomicroscope, in coronal sections that were stained with bromophenol blue.

2.7. Data collection and statistical analysis

We measured T_b, T_{tail}, HR, and Act every 1 min and averaged those values every 10 min. Then, the microdialysate was collected under each condition (before, during, and after cold or heat exposure). The measured parameters are expressed as absolute values (pmol/20 μl). Differences between data were evaluated for statistical significance using repeated measures analysis of variance (ANOVA) with time as the sole factor followed by Bonferroni–Dunn's post hoc tests. Values are expressed as mean ± SEM; $P < 0.05$ was regarded as statistically significant.

3. Results

This study used 24 rats: 16 rats for muscimol/bicuculline studies and 8 rats for measurement of GABA release during cold/heat exposure. Fig. 1 is a schematic representation of the probe tips.

3.1. Effects of muscimol perfusion into PO/AH in a normal environment

Fig. 2 shows that perfusion of muscimol (500 μM) into the PO/AH in a normal environment (23 °C) induced hyperthermia with facilitation of heat production response and heat loss response. Immediately after perfusion of muscimol, HR started to increase. That increase was significant at 80 min and thereafter relative to the baseline (Fig. 2C). In addition, T_b increased after muscimol perfusion; that increase was significant at 90 min and thereafter relative to the baseline (Fig. 2A). T_{tail} increased, but its response was slower than that of either T_b or HR (Fig. 2B).

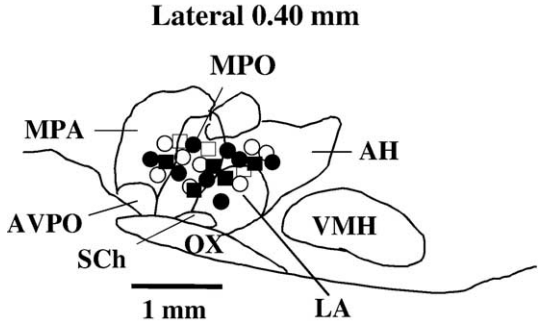

Fig. 1. Schematic representation of a sagittal section showing location of microdialysis probe tips. Respective symbols (●: muscimol study, ○: bicuculline study, ■: cold exposure study, □: heat exposure study) indicate the location of a microdialysis probe implanted into the PO/AH. AVPO, anteroventral preoptic area; AH, anterior hypothalamus; MPA, medial preoptic area; MPO, medial preoptic nucleus; LA, lateral anterior hypothalamus; SCh, suprachiasmatic nucleus; VMH, ventromedial hypothalamus; OX, optic chiasm.

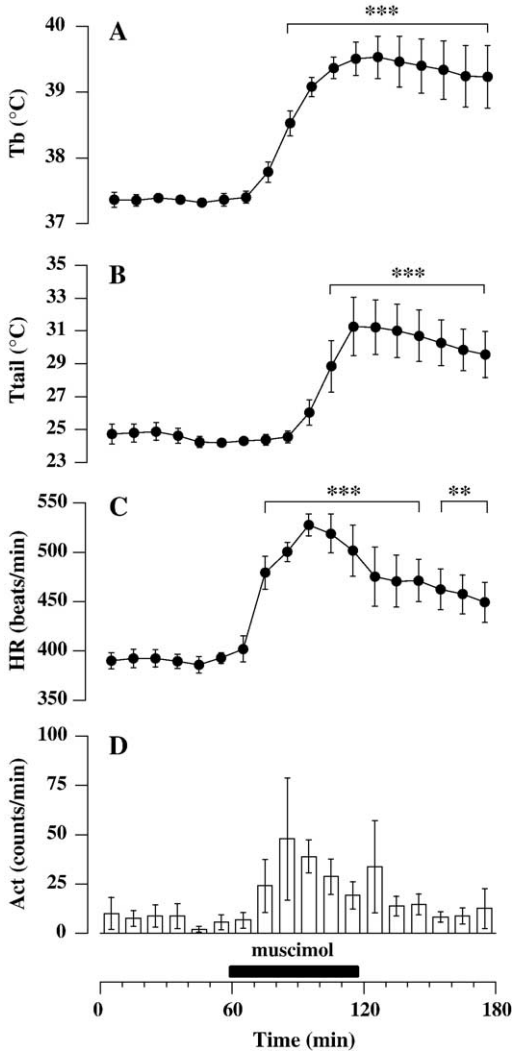

Fig. 2. Changes in T_b (A, $n = 7$), T_{tail} (B, $n = 7$), HR (C, $n = 7$), and Act (D, $n = 7$) elicited by perfusion of the PO/AH with a dialysate containing 500 μM muscimol in a normal environment. Values are expressed as mean ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$ between data within the square bracket and baseline value at 60 min.

Act did not change (47 ± 31 counts/min at 90 min) by perfusion of muscimol (Fig. 2D). The maximum T_b, HR, and T_{tail} were 39.5 ± 0.3 °C (at 130 min), 527 ± 11 beats/min (at 100 min), and 31.2 ± 1.8 °C (at 120 min), respectively.

3.2. Effects of muscimol perfusion into PO/AH in a cold environment

Fig. 3 shows that cold exposure (5 °C) decreased T_{tail} (9.2 ± 0.2 °C at 180 min), increased HR (507 ± 7 beats/min at 140 min), and slightly increased T_b (37.9 ± 0.2 °C at 180 min). However, it did not change Act (41 ± 16 counts/min at 140 min). Perfusion of muscimol (500 μM) into the PO/AH in the cold environment induced further hyperthermia over the steady state during cold exposure with facilitation of heat production responses and a normal heat conservation response. HR and Act increased after perfusion of muscimol. Significant differences were noted respectively from 210 and 220 min (Figs. 3C and D). T_b also began to increase after perfusion of muscimol. That increase was significant at 210 min and thereafter (Fig. 3A). The respective maximum values of T_b, HR, and Act were 39.5 ± 0.1 °C (at 240 min), 575 ± 14 beats/min (at 220 min), and 101 ± 33 counts/min (at 250 min). Perfusion of muscimol did not alter T_{tail} (10.8 ± 0.9 °C at 240 min) (Fig. 3B).

3.3. Effects of muscimol perfusion into PO/AH in a hot environment

Fig. 4 shows that heat exposure (35 °C) induced increases of T_b (39.6 ± 0.2 °C at 180 min), T_{tail} (37.6 ± 0.2 °C at 160 min), and Act (54 ± 16 counts/min at 180 min). HR decreased slightly (357 ± 11 beats/min at 100

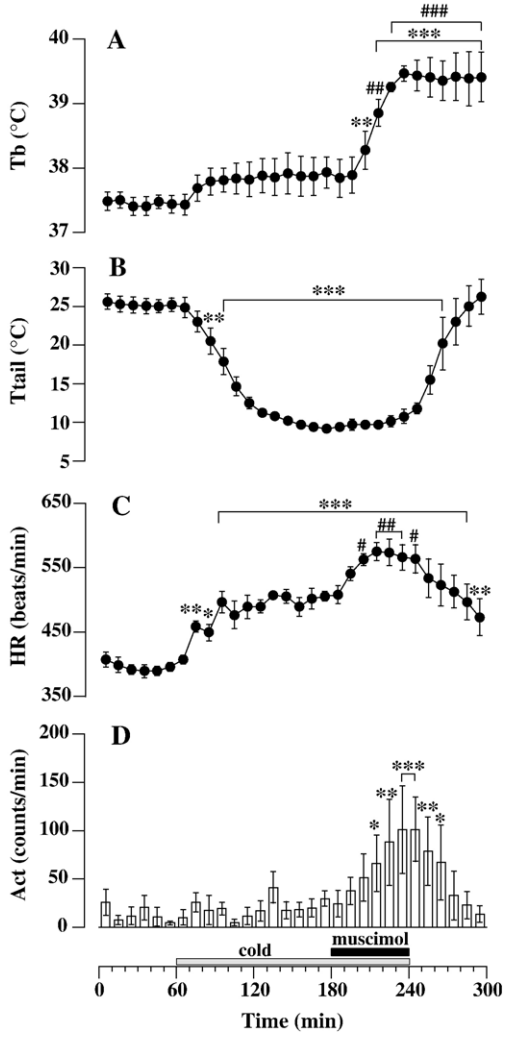

Fig. 3. Changes in T_b (A, $n = 5$), T_{tail} (B, $n = 5$), HR (C, $n = 5$), and Act (D, $n = 5$) elicited by perfusion of the PO/AH with a dialysate containing 500 μM muscimol in a cold environment. Values are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ between data within the square bracket and baseline value at 60 min. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ between data within the square bracket and the steady state value at 180 min.

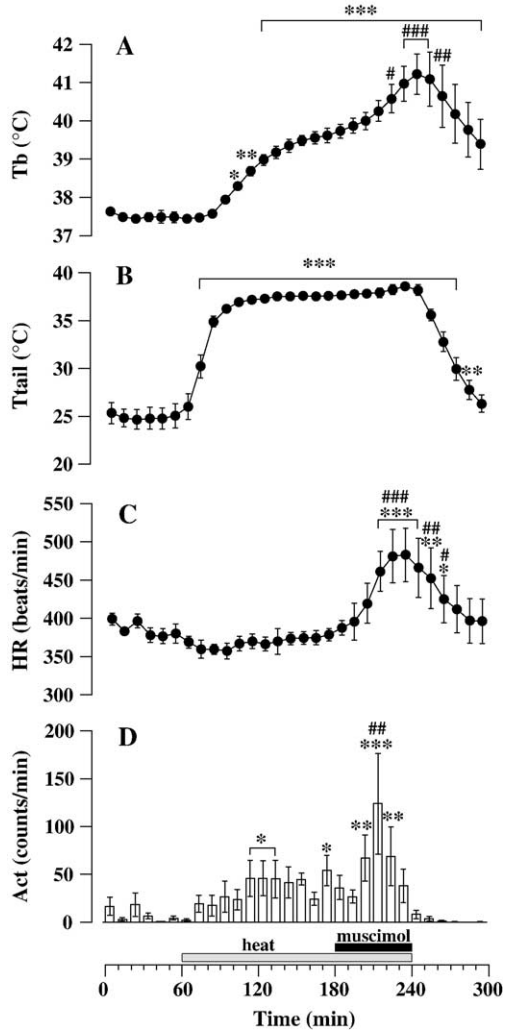

Fig. 4. Changes in T_b (A, $n = 5$), T_{tail} (B, $n = 5$), HR (C, $n = 5$), and Act (D, $n = 5$) elicited by perfusion of the PO/AH with a dialysate containing 500 μM muscimol in a hot environment. Values are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ between data within the square bracket and baseline value at 60 min. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ between data within the square bracket and the steady state value at 180 min.

min), but the difference from the baseline was not significant. These changes stabilized at 60 min after heat exposure. Perfusion of muscimol (500 μM) into the PO/AH with hot ambient temperature induced further hyperthermia over the steady state during heat exposure, with facilitation of heat production response and a minor facilitation of heat loss response. HR increased after perfusion of muscimol. That increase was significant from 220 min (Fig. 4C). T_b also increased immediately after perfusion of muscimol. That increase was significant from 230 min (Fig. 4A). The respective maximum values of T_b and HR were 41.2 ± 0.5 °C (at 250 min) and 483 ± 35 beats/min (at 240 min). T_{tail} (38.6 ± 0.4 °C at 240 min) and Act (124 ± 53 counts at 220 min) increased slightly by perfusion of muscimol compared with their values during heat exposure, but there were no significant differences (Figs. 4B and D).

3.4. Effects of bicuculline perfusion into PO/AH in a normal environment

Figs. 5A–D show that perfusion of bicuculline (50 μM) into the PO/AH under normal environment (23 °C) had no effect on T_b (37.0 ± 0.3 °C at 120 min), T_{tail} (24.6 ± 0.4 °C at 140 min), HR (389 ± 14 beats/min at 140 min), or Act (24 ± 14 counts/min at 90 min).

3.5. Effects of bicuculline perfusion into PO/AH in a cold environment

Fig. 6 shows that cold exposure (5 °C) induced a decrease of T_{tail} (8.9 ± 0.6 °C at 170 min), an increase of HR (507 ± 20 beats/min at 110 min), and a slight increase in T_b (37.7 ± 0.1 °C at 180 min), but no change in Act (50 ± 26 counts/min at 140 min). Perfusion of bicuculline (50 μM) into the PO/AH in a cold environment induced sudden hypothermia in comparison to the steady state during cold exposure with suppression of the heat production response

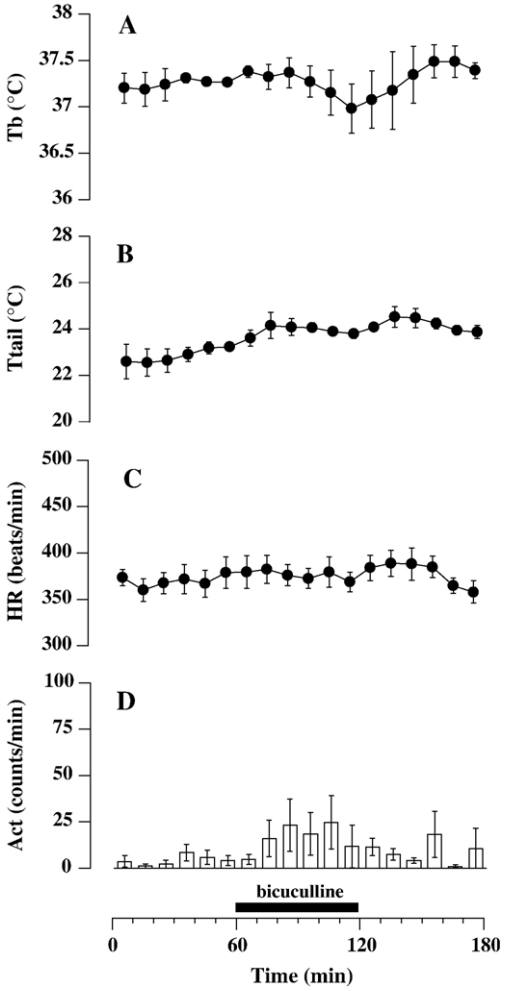

Fig. 5. Changes in T_b (A, $n = 4$), T_{tail} (B, $n = 4$), HR (C, $n = 4$), and Act (D, $n = 4$) elicited by perfusion of the PO/AH with a dialysate containing 50 μM bicuculline in a normal environment. Values are expressed as mean ± SEM.

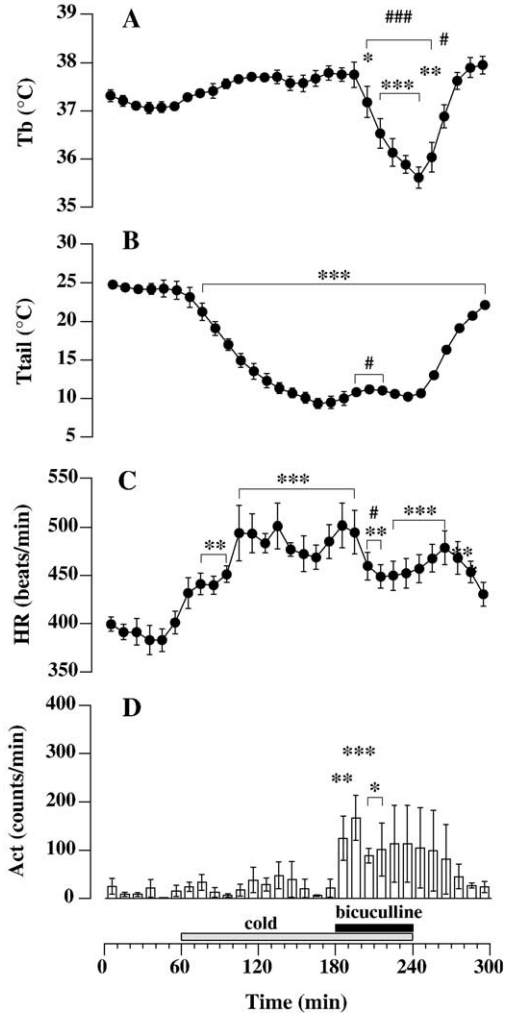

Fig. 6. Changes in T_b (A, $n = 4$), T_{tail} (B, $n = 4$), HR (C, $n = 4$), and Act (D, $n = 4$) elicited by perfusion of the PO/AH with a dialysate containing 50 μM bicuculline in a cold environment. Values are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ between data within the square bracket and baseline value at 60 min. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ between data within the square bracket and the steady state value at 180 min.

and facilitation of heat loss response. HR decreased transiently after perfusion of bicuculline, but that decrease was significant at 220 min (Fig. 6C). T_b began to decrease after perfusion of bicuculline: it was significantly different from the baseline at 210 min and thereafter (Fig. 6A). T_{tail} and Act increased significantly with changes at 200 and 190 min, respectively (Figs. 6B and D). The respective minimum and maximum values of T_b, HR, T_{tail}, and Act were 35.5 ± 0.4 °C (at 230 min), 446 ± 13 beats/min (at 220 min), 11.2 ± 0.5 °C (at 210 min), and 239 ± 40 counts/min (at 200 min).

3.6. Effects of bicuculline perfusion into PO/AH in a hot environment

Fig. 7 shows that heat exposure (35 °C) induced significant increases of T_b (38.8 ± 0.2 °C at 180 min), T_{tail} (37.5 ± 0.5 °C at 180 min), and a decrease of HR (325 ± 14 beats/min at 90 min). However, it did not change Act (12 ± 5 counts/min at 100 min). These changes stabilized at 60 min after heat exposure. Perfusion of bicuculline (50 μM) into the PO/AH under hot ambient temperature induced further hyperthermia over the steady state during heat exposure with facilitation of heat production response and a minor facilitation of heat loss response. HR increased after perfusion of bicuculline. That increase was significantly different at 210 min (Fig. 7C) in comparison with that at 180 min. Nevertheless, no significant difference was seen from the baseline value. T_b also increased immediately after perfusion of bicuculline. That increase was significant from 220 min (Fig. 7A). Respective maximum values of T_b and HR were 39.9 ± 0.3 °C (at 250 min) and 376 ± 8 beats/min (at 230 min). T_{tail} (38.0 ± 0.5 °C at 240 min) and Act (24 ± 7 counts at 200 min) increased slightly by perfusion of muscimol compared with that during heat exposure, but no significant differences were found (Figs. 7B and D).

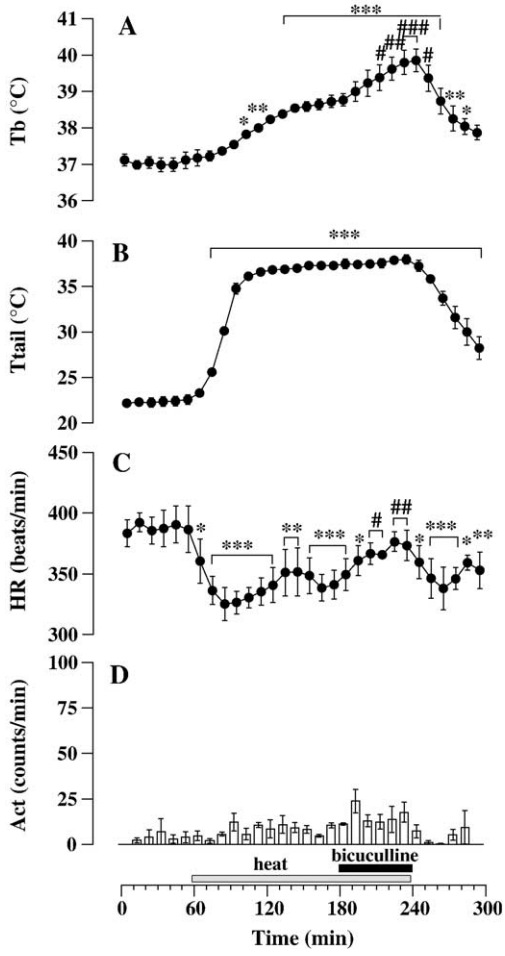

Fig. 7. Changes in T_b (A, $n = 4$), T_{tail} (B, $n = 4$), HR (C, $n = 4$), and Act (D, $n = 4$) elicited by perfusion of the PO/AH with a dialysate containing 50 μM bicuculline in a hot environment. Values are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ between data within the square bracket and the baseline value at 60 min. $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ between data within the square bracket and the steady state value at 180 min.

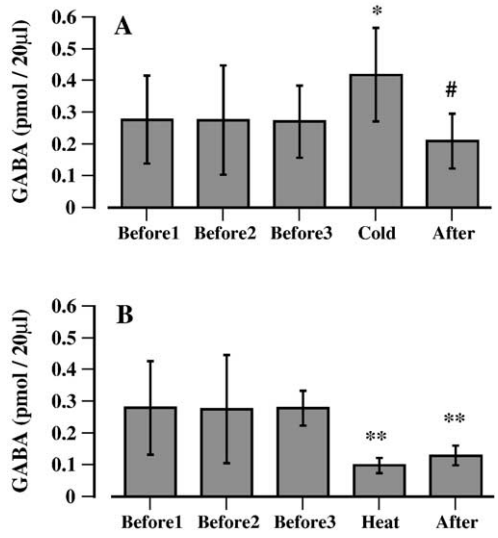

Fig. 8. Changes in extracellular GABA level before, during, and after cold exposure (Before1–3, Cold, After; A, $n = 5$) and before, during, and after heat exposure (Before1–3, Heat, After; B, $n = 3$). Values are expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with Before3. $^{\#}P < 0.05$ compared with Cold.

3.7. Changes in extracellular GABA level in PO/AH during heat or cold exposure

Fig. 8 shows changes in the extracellular GABA level in the PO/AH during cold (5 °C; Fig. 8A) and heat (35 °C; Fig. 8B) exposure. We inferred the value that was shown in a normal environment (23 °C) to be the standard level. Extracellular GABA level significantly increased during cold exposure (0.42 ± 0.15 pmol/20 μl) compared with a normal environment (0.27 ± 0.26 pmol/20 μl), but it returned to the standard level after cold exposure (0.21 ± 0.19 pmol/20 μl). On the other hand, the extracellular GABA level was significantly lower during heat exposure (0.10 ± 0.02 pmol/20 μl) than in a normal environment (0.28 ± 0.06 pmol/20 μl). Its low level was sustained after heat exposure (0.13 ± 0.03 pmol/20 μl).

4. Discussion

This study presents data related to GABAergic system influences in the PO/AH with regard to thermoregulation in freely moving rats. The principle observations are that perfusion of $GABA_A$ agonist muscimol into the PO/AH increased heat production under all ambient temperatures. Perfusion of $GABA_A$ antagonist bicuculline had no effect on thermoregulation at normal ambient temperatures. Furthermore, it inhibited heat production and increased heat loss under cold ambient temperatures. Moreover, it increased heat production under hot ambient temperatures.

The balance between heat production and heat loss regulates T_b. The PO/AH is a major central thermosensitive site for T_b regulation [5,14,18,20]. This site contains both warm- and cold-sensitive neurons that alter their discharge rates corresponding to physiological changes in local temperature or afferent signals from peripheral skin thermoreceptors [4,5,14,20]. It has long been believed that warm-sensitive neurons are important for control of heat loss, whereas cold-sensitive neurons are important for control of heat production [14,20]. However, warm-sensitive neurons in the PO/AH are of three types according to their firing rate [5]. Low and middle firing rate types respectively control heat loss responses such as panting or skin blood flow, whereas the high firing rate type controls heat production [5]. In addition, recent reports have shown that warm-sensitive neurons in the PO/AH mainly contribute to inhibition of heat production [8,29]. 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 increased the heat production response [15].

GABA is known as a predominant inhibitory neurotransmitter in the hypothalamus [3,11]; it is particularly more abundant in the PO/AH than in other brain regions [1,28]. Although GABA reportedly affects both warm-sensitive and cold-sensitive neurons in the PO/AH [17,27], we infer that artificially perfused muscimol into the PO/AH mainly affects warm-sensitive neurons because they are more numerous than cold-sensitive neurons [5,14,20] or are more sensitive to GABA than cold-sensitive neurons [17]. For one or both of those reasons, heat production increased under all ambient temperatures. The observation of increased heat production after muscimol perfusion into the PO/AH is consistent with results of previous studies [21,23]. Previous studies also showed that T_b increases after perfusion of muscimol as a result of activation of heat production response (increase of HR or O_2 consumption) but not heat loss response (no change of cutaneous blood flow or T_{tail}) under the rats' anesthetized condition [21,22]. In this study, T_{tail} only increased after perfusion of muscimol in normal ambient temperature. However, its response was delayed compared with HR and T_b, suggesting that T_{tail} increase was a secondary response to the increased heat production. Warm-sensitive neurons in the preoptic area reportedly excite vasodilatative neurons and inhibit vasoconstrictive neurons in the midbrain [30]. In the present study, perfusion of muscimol might suppress excitatory signals from the PO/AH to vasodilatative neurons in a hot environment. For that reason, no further change in T_{tail} could have occurred because the maximum tail vasodilatation induced by heat exposure had already occurred. In a cold environment, vasoconstriction was inferred to be unaffected because inhibitory signals from the PO/AH could have been inhibited already by cold exposure [5] before muscimol perfusion.

Action of GABA in the PO/AH on thermoregulation was confirmed by perfusion of bicuculline. The reaction of the antagonist was inferred to be different because GABA has

different actions at each environmental temperature. It is reported that peripheral cold stimulation inhibits warm-sensitive neurons in the PO/AH [5]. In addition, this study showed that extracellular GABA level in the PO/AH during cold exposure increases in comparison with normal ambient temperature. Under cold ambient temperatures, GABA largely inhibits warm-sensitive neurons to prevent heat loss and inhibition of heat production. Bicuculline is expected to relieve this inhibition. Therefore, heat loss response might increase and heat production response might therefore decrease in this study. Osaka [21] also showed that bicuculline, injected into the PO/AH, blocked skin-cooling induced thermogenesis. On the other hand, under hot ambient temperatures, GABA largely inhibits cold-sensitive neurons to prevent activation of heat production. Bicuculline should relieve this inhibition, thereby increasing the heat production response. Although the GABA level in the PO/AH under hot ambient temperature was low compared with normal ambient temperatures in this study, we assume that it might have been allowed because the cold-sensitive neurons in the PO/AH are few. Perfusion of bicuculline had no effect on thermoregulation because heat production response is putatively inhibited tonically by warm-sensitive neurons in the PO/AH [8,29] at normal ambient temperatures. Osborne et al. [23] also reported that T_b was not changed after perfusion of bicuculline into the PO/AH in normal ambient temperature (24 °C).

This study demonstrated that GABA in the PO/AH is an important neurotransmitter for disinhibition of heat production and inhibition of heat loss under cold ambient temperatures. It is also a neurotransmitter for inhibition of heat production under hot ambient temperature. A question remains regarding the participation of neurotransmitters other than GABA for thermoregulatory systems in the PO/AH. We recently reported that the possibility of serotonergic systems' participation in the PO/AH for thermoregulation is low [16], but dopaminergic [13], noradrenergic [25], or cholinergic [26] systems in the PO/AH might participate in body temperature regulation. Further studies are necessary to elucidate the thermoregulatory system in the central nervous system.

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