Cold-induced thermogenesis mediated by GABA in the preoptic area of anesthetized rats

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Osaka, Toshimasa. Cold-induced thermogenesis mediated by GABA in the preoptic area of anesthetized rats. Am J Physiol Regul Integr Comp Physiol 287: R306–R313, 2004. First published March 18, 2004; 10.1152/ajpregu.00003.2004.—Bilateral microinjections of GABA (300 mM, 100 nl) or the GABA_A receptor agonist muscimol (100 μM, 100 nl) into the preoptic area (POA) of the hypothalamus increased the rate of whole body O_2 consumption (V\dot{O}_2) and the body core (colonic) temperature of urethane-chloralose-anesthetized, artificially ventilated rats. The most sensitive site was the dorsomedial POA at the level of the anterior commissure. The GABA-induced thermogenesis was accompanied by a tachycardic response and electromyographic (EMG) activity recorded from the femoral or neck muscles. Pretreatment with muscle relaxants (1 mg/kg pancuronium bromide + 4 mg/kg vecuronium bromide iv) prevented GABA-induced EMG activity but had no significant effect on GABA-induced thermogenesis. However, pretreatment with the α-adrenergic receptor antagonist pranolo1 (5 mg/kg iv) greatly attenuated the GABA-induced increase in VO_2 and tachycardic responses. Accordingly, the GABA-induced increase in VO_2 reflected mainly nonshivering thermogenesis. On the other hand, cooling of the shaved back of the rat by contact with a plastic bag containing 28°C water also elicited thermogenic, tachycardic, and EMG responses. Bilateral microinjections of the GABA_A receptor agonist bicuculline (500 μM, 100 nl), but not the vehicle saline, into the POA blocked these skin cooling-induced responses. These results suggest that GABA and GABA_A receptors in the POA mediate cold information arising from the skin for eliciting cold-induced thermogenesis.

thermoafferent; neurotransmitter; nonshivering thermogenesis

THE PREOPTIC AREA (POA) of the hypothalamus is considered to be the primary locus for body temperature regulation, because it integrates thermoafferent signals from the skin and other parts of the body and exerts control over the thermoefferent mechanisms (3, 8, 15, 25). However, the neurotransmitter that mediates thermoafferent signals to the POA is largely unknown, even though there is a great deal of electrophysiological and pharmacological evidence implicating a role for a variety of neurotransmitters, peptides, and cytokines (2, 6, 10, 32). The POA contains GABAergic neurons (27), spontaneously releases GABA to the extracellular space (9, 29), and expresses neurotransmitters, peptides, and cytokines (2, 6, 10, 32).

The POA contains GABAergic neurons (27), spontaneously releases GABA to the extracellular space (9, 29), and expresses GABA_A receptors (7). Perfusion of the POA with the GABA_A agonist muscimol induces hyperthermia, which is not affected by antipyretics and is thus independent of fever, in freely behaving rats (19). Warm-sensitive and thermally insensitive neurons are inhibited by the GABAergic mechanism in the POA (28). Furthermore, it was recently reported that the extracellular GABA level in the POA was increased by acute cold exposure and decreased by heat exposure (14). Therefore, it is possible that GABA is involved in the mechanism of thermoregulation in the POA.

Body temperature is regulated by the balance between heat production and heat dissipation. Therefore, the muscimol-induced hyperthermia (19) can be caused by an increase in heat production or a decrease in heat dissipation. The former can be monitored by the whole body O_2 consumption (V\dot{O}_2) and the latter by the temperature of the tail skin (T_tail). In the present study, the effects of microinjection of GABA and muscimol into the POA on the V\dot{O}_2, T_tail, trunk skin temperature (T_sk), and core temperature (T_c) were investigated in anesthetized rats. Because administration of these agents increased VO_2 without causing significant changes in T_tail, heat production was activated by a GABA-receptive mechanism in the POA. There are two forms of heat production: shivering and nonshivering thermogenesis. The relative contribution of these forms to the GABA-induced thermogenesis was examined by muscle relaxants to block shivering thermogenesis and an adrenergic β-agonist to block nonshivering thermogenesis. Finally, the effects of the GABA_A antagonist bicuculline on skin cooling-induced thermogenesis (17) were examined to elucidate the possible involvement of the GABAergic system in the neurotransmission of thermoafferent signals arising from the skin to the POA.

METHODS

Male Wistar rats, weighing 350–480 g, were maintained at an ambient temperature of 24 ± 1°C with lighting between 0700 and 1900 for ≥1 wk before the experiments. They had free access to water and laboratory food. The care of animals and all surgical procedures followed our institutional guidelines.

After induction of anesthesia with 2–3% isoflurane in air and cannulation of a femoral vein and the trachea, the rats were kept anesthetized intravenously with urethane (600 mg/kg) and α-chloralose (60 mg/kg). An electromyogram (EMG) was recorded with a pair of Teflon-coated flexible stainless steel wires that had been inserted into the dorsal neck or femoral muscles, filtered at 150 Hz–3 kHz, and monitored on an oscilloscope. Occasionally, the signal was digitized at 4 kHz and stored on a hard disk. A mixture of urethane (70–80 mg·kg\(^{-1}\)·h\(^{-1}\)) and chloralose (7–8 mg·kg\(^{-1}\)·h\(^{-1}\)) was continuously administered with the aid of a syringe pump (model 100, KD Scientific) started 90–120 min after the initial anesthesia. Depth of anesthesia was checked by paw pinching, which evoked EMG activity recorded from the same limb but did not elicit withdrawal responses. Pinching the contralateral paw did not evoke EMG activity from the limb measured for the activity. Animals were killed by an overdose of anesthetic at the end of experiment.

The rats were placed in a stereotaxic apparatus with the head fixed according to the coordinate system of Paxinos and Watson (20), and the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
body temperature was maintained at 37–38°C with a heating pad. The back of each rat was shaved between the caudal end of the forelimbs and the rostral end of the hind limbs. Three thermocouples were glued to different sites on the shaved skin. The mean of these three readings was used as the measure of $T_{sk}$. $T_c$ was measured with a fourth thermocouple inserted ~50 mm into the anus. Occasionally, $T_{tail}$ was measured with another thermocouple taped to the dorsal surface of the tail. The trunk and proximal part of limbs were covered with a quilt to reduce heat dissipation.

Respiration was maintained with an artificial respirator (Harvard pump 683). The intermittent expiratory gas from the respirator was introduced into a 30-ml reservoir, which was continuously ventilated with ambient air at a constant rate of 1 l/min. The difference in $O_2$ concentrations between reservoir and ambient air was measured with an $O_2$ analyzer (model LC-700E, Toray). Values were corrected for metabolic body size (kg$^{0.75}$). In some experiments, an electrocardiogram was recorded with needle electrodes subcutaneously inserted into the limbs of the rats and monitored on an oscilloscope. A counter (model AT-601G, Nihon Kohden) was used to detect R waves and calculate heart rate. These signals were fed into a computer and recorded at 3- or 5-s intervals through a PowerLab system (ADInstrum) for online data display, storage, and off-line analysis. After the experiments, data were averaged over 30-s intervals.

A three-barrel glass micropipette was used to apply drugs to the POA or neighboring regions. Each barrel of the pipette contained 300 mM GABA (pH 7.2), 100 μM muscimol hydrobromide (pH 4.5), 500 μM (–)-bicuculline methiodide (pH 5.8), 2% pontamine sky blue, or physiological saline solution. GABA was dissolved in distilled water, and other drugs were dissolved in physiological saline solution. The concentration of the GABA solution was chosen because it is approximately equiosmotic (300 mosmol/kg) to the normal body fluids. The total tip diameter of the pipette was 30–40 μm. The solutions were ejected from the pipette with pressurized nitrogen by the aid of a pressure ejection system (Picospritzer, General Valve) and an eight-way valved connector (model 1103, Omnitit). Injections were made bilaterally. The volume of injected solution was 100 nl on each side. In the case of the injections made into the midline, the volume of solution was also 100 nl. Forjection of the correct amount of a given solution, the displacement of the meniscus between the solution and the nitrogen gas in the pipette was observed through a dissecting microscope with an ocular micrometer. The ejection pressure was adjusted to deliver the solution for 20–30 s on each side.

The β-adrenergic receptor blocker dl-propranolol hydrochloride was dissolved in physiological saline solution and administered at 5 mg/kg iv. A mixture of pancuronium bromide (1 mg/kg) and vecuronium bromide (4 mg/kg) was infused intravenously to paralyze the skeletal muscles. Effects of GABA were examined before and 7–20 min after administration of these drugs.

The method of thermal stimulation of the trunk skin was described previously (17). Briefly, the quilt that covered the rat was removed, and a plastic bag containing 28°C water was placed onto the shaved back for 2 min. The water bag was a 70-μm-thick polyethylene sheet that had a bottom surface area of 110 × 180 mm and contained 340–360 ml of water. The scrotum, tail, head, and distal part of limbs did not receive thermal stimulation. The bag was agitated every 30 s for 4–5 s to mix the water uniformly. The quilt was replaced in its original position soon after the 2-min thermal stimulation.

A volume of 20–30 nl of pontamine sky blue solution was ejected from a barrel of the pipette for histological verification of injection sites at the end of the experiment. The brains were perfused with 10% formalin through the carotid arteries. Coronal sections (40 μm thick) were cut on a freezing microtome, mounted on glass slides, and counterstained with 0.1% neutral red. The sites of injection were identified according to the rat brain atlases of Paxinos and Watson (20) and Swanson (26).

Values are means ± SE. Paired t-test was used to examine the difference in magnitude of GABA- or skin cooling-induced thermogenesis before and after administration of drugs. Statistical significance was defined as $P < 0.05$.

**RESULTS**

Figure 1 shows a representative example of the effects of skin cooling and microinjection of GABA, muscimol, or physiological saline into the medial POA of a rat. Skin cooling increased $V_O_2$ and heart rate and temporarily decreased $T_c$, effects similar to those observed previously (17). The micro-injection of GABA increased $V_O_2$ during or within 30 s after the injection by $3.62 ± 0.56$ ml·kg$^{-0.75}$·min$^{-1}$ ($n = 15$) at $5.0 ± 0.5$ min, and then $V_O_2$ returned to the baseline level within 20 min. The GABA injection also increased heart rate by $56 ± 13$ beats/min at $4.2 ± 0.5$ min ($n = 13$) and $T_c$ by $0.27 ± 0.04°C$ ($n = 15$) at 8–22 min after the injection. $T_{sk}$ was $36–38°C$ and increased by $0.25 ± 0.04°C$ after the GABA injection with a time course similar to that of $T_c$. On the other hand, injection of muscimol increased $V_O_2$ by $4.59 ± 0.34$ ml·kg$^{-0.75}$·min$^{-1}$ ($n = 6$) for >40 min (Fig. 1B and 2C). The muscimol injection increased $T_c$ by $0.71 ± 0.14°C$ and $T_{sk}$ by $0.85 ± 0.23°C$ at 20–50 min after injection. $T_{tail}$ was $29–32°C$ and increased by 0.3–1.5°C after the muscimol injection ($n = 3$; Fig. 2F), although it did not change significantly after the GABA injection ($n = 6$; see Fig. 5E). Injection of physiological saline had no effect on $V_O_2$, heart rate, $T_c$, or $T_{sk}$ ($n = 8$).

The thermogenic effect of GABA and muscimol was restricted to an area in and around the dorsomedial POA. Figure 2, A and B, shows specific sensitivity of the dorsomedial POA to GABA and the lack of a thermogenic response by the ventromedial, dorsolateral, or ventrolateral POA. Similarly, micro-injection of muscimol into the dorsomedial POA, but not into the anterior commissure or ventromedial POA, elicited a thermogenic response (Fig. 2, C and D). Figure 3 shows the site of GABA or muscimol microinjection and the $V_O_2$ responses. The most effective injection sites were clustered in the dorsomedial POA at the level of the anterior commissure, although small responses were observed when microinjection was made into the dorsolateral POA and vertical limb of the diagonal band of Broca. Injection of GABA into the median, periventricular, or ventral preoptic area had no effect on $V_O_2$.

Microinjection of GABA or muscimol usually elicited EMG activity recorded from the femoral or neck muscles. The GABA-induced EMG activity started simultaneously with or up to 3 min after the rise in $V_O_2$ and lasted $8.0 ± 1.2$ min ($n = 11$). The EMG consisted of several different-sized action potentials (Fig. 4C), suggesting concurrent activation of a limited number of muscle fibers. The discharge pattern was continuous and did not exhibit the rhythmic burst that is typically observed during shivering. Despite the EMG activity, however, the rat did not exhibit any visible body movement. Because the result could suggest a contribution of muscle contractions to the GABA-induced thermogenesis, the effects of the muscle relaxants were examined. Administration of the muscle relaxants alone had various transient (<5 min) and small (<1 ml·kg$^{-0.75}$·min$^{-1}$) effects on $V_O_2$: it was decreased ($n = 5$), increased ($n = 3$), or without effect ($n = 1$). However, the GABA-induced EMG activity was blocked effectively (cf. Fig. 4, C and D), whereas the thermogenic response to GABA largely persisted (Fig. 4, A and B). Figure 4E summarizes the increase in $V_O_2$ in individual rats before and after administration of the muscle relaxants ($n = 9$). Although the response...
Administration of bicuculline alone had variable effects on skin cooling-induced thermogenesis and microinjection of GABA, muscimol, and physiological saline into the preoptic area (POA) in a rat. Horizontal bars in B show period of microinjection.

Effects of the microinjection of the GABA A receptor antagonist bicuculline into the POA on skin cooling and microinjection of GABA, muscimol, and physiological saline into the preoptic area (POA) in a rat. Horizontal bars in B show period of microinjection.

**DISCUSSION**

Microinjection of GABA or muscimol into the POA increased VO2 and Tc, indicating a thermogenic response mediated by a GABA-receptive mechanism in the POA. The hyperthermic response agrees well with the results of a previous study on conscious rats (19). Because GABA is an inhibitory neurotransmitter, these results suggest that the GABA-recep-
tive mechanism in the POA tonically suppresses thermogenesis and that GABA-induced inhibition of the POA neurons activated thermogenesis by disinhibition of this system. Consistent with this notion, Chen et al. (5) demonstrated an inhibitory influence of preoptic neurons on nonshivering thermogenesis: microinjection of the excitatory amino acid DL-homocysteic acid into the POA attenuated nonshivering thermogenesis elicited by electrical stimulation of the ventromedial hypothalamus. Similarly, perfusion of the POA with tetrodotoxin induced hyperthermia and tachycardia in conscious rats, suggesting an inhibitory function of the POA with respect to heat production (13). On the other hand, administration of GABA in the present study had no significant effect on T_{tail}, which was within the baseline level of 29–32°C. These effects on T_{tail} suggest that the tail skin was in a steady vasoconstrictive state and did not exhibit a further vasomotor response to GABA. The GABA-induced thermogenesis was greatly attenuated by pretreatment with the β-blocker propranolol but not significantly by pretreatment with the muscle relaxants. These results indicate that mainly nonshivering thermogenesis was elicited in the present study. However, they do not exclude the possibility of an involvement of preoptic GABA-receptive neurons in the mechanism of shivering thermogenesis. Microinjection of GABA or muscimol did evoke EMG activity, and pretreatment with bicuculline prevented the skin cooling-induced EMG activity in the present study. The POA reportedly regulates shivering and nonshivering thermogenesis (3, 5, 33). Accordingly, it is possible that GABA is also involved in the

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**Fig. 2.** Site-specific thermogenic effect of GABA and muscimol. Representative records show site-specific actions of GABA (A and B) and muscimol (C–F) on \( V_{O2} \) (A and C), \( T_c \) (B and D), \( T_v \) (E), and tail skin temperature (\( T_{tail} \), F). Note different time scale in A and B vs. C–F. Horizontal bars show period of microinjection. DMPO, dorsomedial preoptic area; VMPO, ventromedial preoptic area; DLPO, dorsolateral preoptic area; VLPO, ventrolateral preoptic area; AC, anterior commissure.
increase in skeletal muscle tone during shivering thermogenesis, although the relative contribution of shivering to heat production was small under the present experimental conditions.

The GABA-sensitive site for thermogenesis was localized in or near the dorsomedial POA at the level of the anterior commissure. The POA is composed of several histologically distinct subdivisions (24). Among these subdivisions, the ventromedial area has been proposed to participate in hyperthermia during fever (21, 22), and the ventrolateral area is particularly involved in the regulation of sleep (23). However, no report has identified any specific thermoregulatory function for
the dorsomedial area, although thermosensitive neurons (11, 16) and GABA binding sites (1) were found to be distributed diffusely in and around the medial POA.

$V_O^2$ and heart rate changed roughly in a parallel fashion in response to the GABA injection and various other treatments employed in the present study, suggesting a common GABA-receptive mechanism for the thermogenic and tachycardic responses in the POA. However, although administration of the GABA$_A$ antagonist bicuculline alone always decreased the basal heart rate, it did not produce a consistent effect on $V_O^2$.

These results suggest that the GABA-receptive mechanism for the control of thermogenesis is not totally identical to that governing heart rate and that the spontaneous release of GABA in the POA contributed to the basal heart rate but not significantly to $V_O^2$ under the present experimental conditions. On the other hand, β-adrenoceptors are critically involved in the peripheral mechanism of the GABA-induced thermogenic and tachycardic responses, because systemic administration of propranolol greatly attenuated both responses. Consistent with the present results, tachycardia induced by perfusion of the POA with muscimol was blocked by systemic administration of propranolol or adrenalectomy in halothane-anesthetized rats (18).

Local cooling of the POA reportedly increased $V_O^2$ and temperature of the brown adipose tissue in conscious rats, suggesting activation of nonshivering thermogenesis (12). The POA contains two types of thermosensitive neurons: cold-sensitive neurons are excited, and warm-sensitive neurons are inhibited, by local brain cooling. Accordingly, it is likely that the preoptic cooling-induced thermogenesis was mediated by excitation of the cold-sensitive neurons or inhibition of the warm-sensitive neurons. Because GABA inhibits spontaneous activity of warm-sensitive neurons in the POA (28), we may surmise that the GABA-induced thermogenesis was mediated by inhibition of warm-sensitive neurons, rather than by excitation of cold-sensitive neurons.
Thermogenic responses were elicited by skin cooling as well as by microinjection of GABA or muscimol into the POA. Skin cooling-induced thermogenesis, tachycardia, and EMG activity were blocked by microinjection of bicuculline into the POA. These results demonstrate that GABA and GABAA receptors in the POA exert a pivotal role in skin cooling-induced thermogenesis. Peripheral cold stimulation inhibits warm-sensitive neurons in the POA (4). Therefore, it may be inferred that cold information originating from the skin activates GABAergic fibers that terminate on warm-sensitive neurons in the POA, although further studies are necessary to verify this possibility. Alternatively, it is also possible that the GABA-receptive mechanism in the POA tonically inhibits some other key locus that receives the cold signal from the skin and has an excitatory effect on heat production. The dorsomedial hypothalamus has been suggested as such a locus (30).

Although the present study demonstrates the critical involvement of the GABA-receptive mechanism in the POA in cold-induced thermogenesis, the participation of neurotransmitters other than GABA cannot be excluded in the thermoafferent system. For example, serotonergic and noradrenergic systems have been proposed to mediate or modulate the afferent signals in the central thermoregulatory pathways (8, 31), although no definite evidence has been demonstrated. GABA and other
transmitters can function in parallel or in series to mediate thermal information, which affects various thermoregulatory functions, such as shivering and nonshivering thermogenesis, cutaneous vasoconstriction, and behavioral thermoregulation. Further studies are necessary to elucidate the relations among neurotransmitters operating in the various thermoregulatory subsystems. Moreover, the location and properties of the GABAergic neurons that mediate thermogenesis remain to be clarified.

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REFERENCES