Inhibition of the preoptic area and anterior hypothalamus by tetrodotoxin alters thermoregulatory functions in exercising rats

Hiroshi Hasegawa, Takayuki Ishiwata, Takehito Saito, Toru Yazawa, Yasutsugu Aihara, and Romain Meeusen. Inhibition of the preoptic area and anterior hypothalamus by tetrodotoxin alters thermoregulatory functions in exercising rats. J Appl Physiol 98: 1458–1462, 2005. First published December 23, 2004; doi:10.1152/japplphysiol.00916.2004.—We have previously demonstrated a functional role of the preoptic area and anterior hypothalamus (PO/AH) in thermoregulation in freely moving rats at various temperature conditions by using microdialysis and biotelemetry methods. In the present study, we perfused tetrodotoxin (TTX) solution into the PO/AH to investigate whether this manipulation can modify thermoregulation in exercising rats. Male Wistar rats were trained for 3 wk by treadmill running. Body core temperature (Tb), heart rate (HR), and tail skin temperature (Ttail) were measured. Rats ran for 120 min at speed of 10 m/min, with TTX (5 μM) perfused into the left PO/AH during the last 60 min of exercise through a microdialysis probe (control, n = 12; TTX, n = 12). Tb, HR, and Ttail increased during the first 20 min of exercise. Thereafter, Tb, HR, and Ttail were stable in both groups. Perfusion of TTX into the PO/AH evoked an additional rise in Tb (control: 38.2 ± 0.1°C; TTX: 39.3 ± 0.2°C; P < 0.001) with a significant decrease in Ttail (control: 31.2 ± 0.5°C, TTX: 28.3 ± 0.7°C; P < 0.01) and a significant increase in HR (control: 425.2 ± 12 beats/min, TTX: 502.1 ± 13 beats/min; P < 0.01). These results suggest that the TTX-induced hyperthermia was the result of both an impairment of heat loss and an elevation of heat production during exercise. We therefore propose the PO/AH as an important thermoregulatory site in the brain during exercise.

Address for reprint requests and other correspondence: H. Hasegawa, Dept. of Human Physiology and Sportsmedicine, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium (E-mail: hhasegaw@vub.ac.be).

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IT IS ESTABLISHED THAT EXERCISE performance is impaired in high ambient temperature (10, 15, 39). Interestingly, exhaustion during prolonged exercise in the heat appears to coincide with the attainment of a critical internal body temperature (Tb) of ~40°C (12, 34). The attainment of a critically high body core temperature during prolonged exercise has been suggested to result in a loss of central nervous system drive (33, 35), and it has been associated with increased perception of effort (36) and with altered electroencephalographic brain activity of the frontal cortex (34). As a result, high body core temperature is proposed as a major factor limiting endurance performance in both human (8, 12, 14, 35) and animal studies (9, 43). To successfully improve exercise performance to maintain Tb below the critical limits in hot conditions (26), several strategies, including heat acclimatization (8, 34), precooling (2, 24), fluid ingestion (27, 29), and a combination of two of these methods (16), have been investigated. However, the exact mechanisms for these hyperthermia-induced effects and how thermal stress affects brain neurotransmission during exercise are not well understood.

The preoptic area and anterior hypothalamus (PO/AH) are thought to be the primary locus for regulation (1, 4, 23) due to the fact that the PO/AH contains both warm-sensitive and cold-sensitive neurons that respond to small changes in temperature (3, 20, 25, 31, 32). Thereby, this brain area integrates thermal information from central and peripheral thermoreceptors, and it initiates appropriate heat loss and heat production responses (1, 4, 23). Moreover, local warming of PO/AH induces heat loss, whereas cooling of this area results in enhancement of heat production (1, 3), and lesions made in the bilateral PO/AH have been demonstrated to produce a severe impairment in thermoregulation (19).

Our laboratory has recently investigated the functional role of the PO/AH in thermoregulation in freely moving rats using microdialysis and biotelemetry methods (21). Tetrodotoxin (TTX), a poison of the Japanese puffer fish that acts as a sodium- channel blocker, is widely used for blockage of neurotransmission in specific brain regions. When employed together with brain microdialysis, this method can be used to elucidate possible mechanisms of neurotransmitter action (5, 44, 45). The perfusion of TTX into the PO/AH induces hyperthermia (21). This response is accompanied by an increase in heat production with no change in heat loss under temperate environmental conditions (23°C) and during heat exposure (35°C) (21). These results support previous findings that warming of the PO/AH (i.e., activation of warm-sensitive neurons) suppresses shivering (47) and that chemical stimulation with microinjection of the excitatory amino acid D,L-homocysteic acid into the PO/AH attenuated nonshivering thermogenesis by electrical stimulation of the ventromedial hypothalamus (7). These results suggest that, in resting animals, the PO/AH primarily acts as an inhibitory control against other loci that regulate heat production responses. However, these arguments do not appear to be consistent with the functional role of the PO/AH in thermoregulation during exercise. Because exercise significantly increases heat production, the PO/AH area may also activate heat loss mechanisms (18). Moreover, the dissipation of heat from the body is thought to be more important to the regulation of Tb during exercise than the control of heat production (11). In exercising rodents, tail skin vasoconstriction is
an important route of heat loss from the body, because rats do not dissipate heat though the evaporation of sweat. Furthermore, it is unlikely that behavioral heat loss responses, such as evaporative cooling by spreading saliva onto the skin and fur, are involved while the animal is exercising (41). In addition, it is known that PO/AH cell groups project to the sympathetic outflow of the tail artery involved in heat loss in the rat (42).

The purpose of the present study was to examine thermoregulatory functions of the PO/AH in exercising rats by infusing TTX, a neurotoxin, to inhibit neurotransmission in this brain region. Brain microdialysis and biotelemetry were employed to reduce the stress of pharmacological stimulation (28) and the manipulation in measuring Tb (13). Exercise was used to facilitate thermoregulatory responses such as heat production and heat loss. We hypothesized that infusion of TTX through the microdialysis probe into the thermoregulatory center would inhibit the heat loss responses and accelerate the heat production during prolonged exercise.

**MATERIALS AND METHODS**

Animal treatment and experimental procedures. Male Wistar rats (300–350 g at the start of the experiment) were used in all experiments. Animals were housed separately in plastic cages under controlled conditions of ambient temperature (23°C) on a 12:12-h light-dark cycle (lights on at 0600). Animals had a standard diet with free access to food and water through the 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.

Exercise familiarization sessions and surgery. The animals were exercised once a day, 4 days/wk, for 3 wk using a rodent treadmill. The running time and speed gradually increased on a daily basis. After the training protocol, the rats that ran naturally without the use of electric shock were selected for telemetry implantation surgery (17). A telemetry device (TA10TA-F20 or TA10ETA-F20, Data Sciences International) was implanted in the peritoneal cavity under Nembutal anesthesia (50 mg/kg ip). After surgery, the rats were given at least 6 days of recovery. This was followed by a treadmill readaptation period (1 wk), and 2 days before experiments the rats were anesthetized with Nembutal (50 mg/kg ip) to implant the microdialysis probe (0.24-mm external diameter, 2.0-mm-long dialyzing membrane, model CUP 11, CMA Microdialysis, Solna, Sweden) in the left lateral PO/AH (anteroposterior −0.4 mm; lateral +0.5 mm; dorsal −8.3 mm) (40). The probe was secured to the skull using dental cement.

**Experimental protocol.** Rats were randomly divided into two treatment groups (Ringer control and TTX). On the day of the experiments, the microdialysis probe was connected to a microinjection pump (model CMA 100, CMA Microdialysis) and was perfused with a modified Ringer solution (147 mmol/l NaCl, 4 mmol/l KCl, and 2.3 mmol/l CaCl2) at a flow rate of 1 μl/min.

The rats were first monitored resting in their home cages for 60 min before resting on the treadmill for 120 min to verify stable basal conditions. The animal was then exercised at a moderate speed of 10 m/min on motor-driven horizontal treadmill for 120 min. An adjustment was made to treadmill to attach the counterbalance arm of the microdialysis system (28).

In the TTX group, TTX solution (5 μM) was perfused during the last 60 min of exercise using a microdialysis probe and liquid switch (model CMA/110, CMA Microdialysis) (21). Recovery from exercise was monitored on the treadmill for 120 min and then in their home cages for an additional 60 min.

Tb and heart rate (HR), indicator of heat production (6, 21, 30), were simultaneously monitored by using a biotelemetry system every minute, and the data were averaged every 5 min. Tail skin temperature (Ttail), an index of heat loss responses (18, 21), was measured at 1-min intervals on the dorsal surface of the skin −10 mm from the base of the tail using an by alumel-chromel thermocouple (21). The thermocouple was attached with tape just before the beginning of exercise. The ambient temperature was kept at 23°C throughout the study.

Histological examination. At the end of each experiment, rats were killed with an overdose of Nembutal (100 mg/kg ip). The placement of the microdialysis probe was verified in coronal sections stained with bromophenol blue (18) according to the coordinates described by Paxinos and Watson (40).

**Statistical analysis.** Data are presented as means ± SE. Differences between data were evaluated for statistical significance by using a two-factor (time and conditions) repeated-measures ANOVA followed by Bonferroni/Dunn post hoc tests. P < 0.05 was regarded as statistically significant.

**RESULTS**

We performed successful experiments on both control and TTX groups (control: n = 12, TTX: n = 12). As shown in Fig. 1, the tip of the microdialysis probe was correctly positioned into the PO/AH in all these animals.

Changes in Tb are presented in Fig. 2. The handling stress caused by moving the rats from their home cages to the
treadmill produced a transient increase in $T_b$ (at $-120$ min). Before the start of exercise, $T_b$ was stable for at least 60 min. A progressive elevation in $T_b$ was apparent during first 20 min of exercise. Thereafter, $T_b$ was maintained at a steady high level during exercise in both groups (control: $38.2 \pm 0.1^\circ C$, TTX: $38.2 \pm 0.1^\circ C$, the average from 30 to 60 min). Perfusion of TTX into the PO/AH (from 60 to 120 min) influenced $T_b$, with values significantly elevated in the TTX trial (TTX: $39.3 \pm 0.2^\circ C$ at 110 min) above those recorded in control trial ($38.2 \pm 0.1^\circ C$ at 110 min) ($P < 0.001$). These differences in $T_b$ between control and TTX conditions were maintained throughout the remainder of exercise. Exercise behavior, which was determined through subjective observations of the rodents in both control and TTX trials, did not differ. During recovery, $T_b$ gradually decreased; however, $T_b$ was significantly higher in the TTX group, even 3 h after perfusion of TTX, than in the control group. As a result of the handling (placing the animals back in the home cage), $T_b$ increased again at 240 min in both treatment groups.

Although HR immediately increased at $-120$ min due to the handling stress, it decreased and was stable for at least 60 min before the start of exercise (Fig. 3). Treadmill exercise elevated HR during the first 20 min, before reaching a stable level in both groups (control: $421.5 \pm 1.6$ beats/min, TTX: $412.3 \pm 1.6$ beats/min, the average from 30 min to 60 min). HR increased after TTX perfusion (control: $425.2 \pm 12$ beats/min, TTX: $502.1 \pm 13$ beats/min at 110 min), and it was significantly different between two conditions from 80 min to the end of exercise ($P < 0.05$). During the recovery period, HR immediately decreased, but HR was significantly higher in the TTX group, even 2 h after perfusion of TTX, than in the control group. As a result of the handling, HR increased again at 240 min.

$T_t$ increased within 15 min of exercise in both conditions (Fig. 4), indicating that vasodilation had occurred and showing activation of heat loss mechanisms. Thereafter, $T_t$ remained stable in both groups (control: $31.7 \pm 0.1^\circ C$, TTX: $31.4 \pm 0.1^\circ C$, the average from 30 to 60 min). $T_t$ decreased rapidly from the onset of perfusion of TTX (control: $31.2 \pm 0.5^\circ C$, TTX: $28.3 \pm 0.7^\circ C$ at 110 min), and a significant difference between two conditions was found from 65 min ($P < 0.05$) until the end of exercise. During the recovery period, $T_t$ gradually decreased; however, a difference in $T_t$ was apparent between conditions until 170 min.

Perfusion of TTX in other brain regions produced no significant effect on thermoregulatory responses to exercise ($n = 7$). For example, when TTX was induced in the posterior hypothalamus, which is considered to be involved in shivering thermogenesis especially in the cold environment, no effect on the $T_b$, HR, and $T_c$ was seen during exercise. Moreover, we also did not observe any change in the thermoregulatory responses to exercise after TTX infusion into the fornix and dorsal hypothalamic area. However, a small increase in $T_b$ during the latter stages of exercise was observed when TTX perfusion was made into the third ventricle.

**DISCUSSION**

The main finding of the present study was that perfusion of TTX into the PO/AH during treadmill exercise induced an increase in $T_b$ with a decrease in heat loss responses and an increase in heat production. The TTX-induced hyperthermia was not accompanied with any change in exercise behavior. Moreover, alteration in thermoregulatory responses by perfusion of TTX was restricted to the PO/AH. To the best of our knowledge, this is the first study to examine the effect of TTX infusion into the brain on the thermoregulatory responses to prolonged exercise.

A number of resting studies have measured $T_b$ changes after the introduction of TTX, a sodium-channel blocker, into the hypothalamus. Jones et al. (22) reported that microinjection of TTX into the anterior hypothalamus resulted in an increase of $T_b$ in conscious cats. Osborne et al. (38) also reported that perfusion of TTX by microdialysis into the PO/AH elevated brain temperature in conscious freely moving rats. Westerink et al. (46) described a procedure in which infusion of TTX during brain microdialysis was proposed as a method for characterizing voltage-dependent neurotransmitter release.
Recent work from our laboratory has investigated the effect of TTX perfusion into the PO/AH on thermoregulation in conscious freely moving rats. In that study, our laboratory examined the possible thermoregulatory effector mechanisms. It was found that the TTX-induced hyperthermia was due to an increase in heat production mechanisms because HR was increased and heat loss response was not influenced by TTX perfusion in both temperate environmental condition (23°C) and heat exposure (35°C) (21). The results of the present study indicate that perfusion of TTX into the PO/AH elevated the Tb and HR during exercise. This is consistent with the responses reported in the above mentioned studies (Figs. 2 and 3). Recent findings by Kanosue and colleagues (7, 47) demonstrated that electrical and chemical stimulation of neurons in the preoptic area (POA) attenuated shivering and nonshivering thermogenesis in anesthetized rats. In addition, Osaka (37) has also recently reported that microinjection of GABA or muscimol, a GABA_A receptor agonist, into the POA increased thermogenic responses. Because GABA is an inhibitory neurotransmitter, the GABA-receptive mechanism in the POA tonically suppresses thermogenesis; therefore, the GABA-induced inhibition of the POA neurons activated thermogenesis by inhibition of this system. For the reasons mentioned above, their results and our findings suggest that the functional role of the PO/AH in heat production system is an inhibitory control, possibly regulating other loci that are involved in heat production responses. In other words, inhibition of the PO/AH neurons by perfusion of TTX activated the disinhibition of thermogenesis of this system.

Because the previous results are not consistent with a pivotal role of the PO/AH in thermoregulation during exercise, we performed the present study. We hypothesized that if the PO/AH is not only involved in inhibitory role of heat production, but also heat loss responses during exercise, TTX would act on both the inhibition of heat loss responses and an increase in heat production (possibly through an upregulation of heat production). In the present study, inhibition of PO/AH neurons by perfusion of TTX during exercise induced an additional increase in Tb (Fig. 2) with not only an enhancement of heat production mechanisms because HR was increased and heat loss response was not influenced by TTX perfusion in both temperate environmental condition (23°C) and heat exposure (35°C) (21). The results of the present study indicate that perfusion of TTX into the PO/AH elevated the Tb and HR during exercise. This is consistent with the responses reported in the above mentioned studies (Figs. 2 and 3). Recent findings by Kanosue and colleagues (7, 47) demonstrated that electrical and chemical stimulation of neurons in the preoptic area (POA) attenuated shivering and nonshivering thermogenesis in anesthetized rats. In addition, Osaka (37) has also recently reported that microinjection of GABA or muscimol, a GABA_A receptor agonist, into the POA increased thermogenic responses. Because GABA is an inhibitory neurotransmitter, the GABA-receptive mechanism in the POA tonically suppresses thermogenesis; therefore, the GABA-induced inhibition of the POA neurons activated thermogenesis by inhibition of this system. For the reasons mentioned above, their results and our findings suggest that the functional role of the PO/AH in heat production system is an inhibitory control, possibly regulating other loci that are involved in heat production responses. In other words, inhibition of the PO/AH neurons by perfusion of TTX activated the disinhibition of thermogenesis of this system.

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Not only is it possible to monitor changes in neurotransmitters using the microdialysis probe but it is also possible to infuse drugs through the cannula. This technique is widely used in the area of microdialysis, and it enables one to study the effect of a drug in a very restricted region (5). However, it is important to know how drugs diffuse throughout the brain from the microdialysis probe. Westerink and De Vries (45) measured quantitatively the diffusion rate of the infused drugs (such as dopamine agonist, dopamine-reuptake inhibitor, and TTX) from a microdialysis probe across the brain tissue using a dual-probe microdialysis method. They reported that 1 μM TTX acts to block neural activity over a radius of 1 mm in the striatum and that the penetration rate of TTX was faster than other drugs. Although there is a regional difference between the hypothalamus and striatum, TTX may also act on the surrounding 1 mm of the probes in the hypothalamus. This suggests that TTX blocks the neural signals in most areas of the PO/AH but not outside of the PO/AH. Moreover, from a viewpoint of specific neuronal characteristics, it might be suggested that TTX-induced hyperthermia could be the result of impairment of warm-sensitive neurons activity during exercise rather than the cold-sensitive neurons. It has been shown that there are more warm-sensitive neurons than cold-sensitive neurons in the PO/AH (4).

Because TTX does not block one specific neurotransmitter system, further research necessary to elucidate the role of specific neurotransmitters in the control of thermoregulation during exercise.

Conclusion. Perfusion of TTX into the PO/AH induced a significant increase in Tb with a significant decrease in Ttail and an elevation in HR. The TTX-induced hyperthermia was found without a change in exercise behavior. Moreover, alteration in thermoregulatory responses by perfusion of TTX was restricted to the PO/AH. These results indicate that TTX-induced hyperthermia results from both the impairment of heat loss and an enhancement of heat production during exercise, and that the PO/AH is the critical thermoregulatory site in the brain under these conditions.

ACKNOWLEDGMENTS

We thank Dr. M. Yasumatsu for the valuable discussion of the study and Dr. P. Watson for English revision of the manuscript.

GRANTS

This work was supported in part by Ministry of Education, Science and Culture of Japan for Scientific Research Grant 14780012.

REFERENCES

ROLE OF PO/AH IN EXERCISING RATS


