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The median preoptic nucleus is involved in the facilitation of heat-escape/cold-seeking behavior during systemic salt loading in rats

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Konishi, Masahiro, Kazuyuki Kanosue, Masumi Kano, Akiko Kobayashi, and Kei Nagashima. The median preoptic nucleus (MnPO) would be involved in this mechanism. Rats were divided into two groups (n = 6 each): one group had the MnPO lesion with ibotenic acid (4.0 µg) and the other was the vehicle control. After subcutaneous injection (10 ml/kg) of either isotonic- (154 mM) or hypertonic-saline (2,500 mM), each rat was placed in a behavior box, where the ambient temperature was changed to 26°C, 35°C, and 40°C every 1 h. The position of a rat in the box and the body core temperature were greater (P < 0.05) in the 35°C and 40°C heat when moved in a specific area in the box (operant behavior). In the control group, counts of the operant behavior were greater (P < 0.05) in the hypertonic- than in the isotonic-saline injection (17 ± 2 and 10 ± 2 at 35°C, 24 ± 2 and 18 ± 1 at 40°C). T-core remained unchanged throughout the exposure, although the level was lower (P < 0.05) in the hypertonic- than in the isotonic-saline trial (36.6 ± 0.2°C and 37.4 ± 0.1°C at 26°C and 36.9 ± 0.2°C and 37.4 ± 0.1°C at 40°C, respectively). However, in the MnPO-lesion group, counts of the behavior were similar between the hypertonic- and isotonic-saline injection trials (10 ± 2 and 8 ± 1 at 35°C, and 17 ± 1 and 16 ± 1 at 40°C, respectively). T-core increased (P < 0.05) in the heat in both trials (36.8 ± 0.1°C and 37.4 ± 0.1°C at 26°C and 37.4 ± 0.2°C and 37.8 ± 0.2°C at 40°C in the hypertonic- and isotonic-saline injection trials, respectively). These results may suggest that, at least in part, the MnPO is involved in the facilitation of heat-escape/cold-seeking behavior during osmotic stimulation.

osmolality; body temperature; operant behavior; lesion

IT HAS BEEN REPORTED THAT increases in osmolality and Na⁺ concentration in the plasma and/or cerebrospinal fluid (CSF) modulate thermoregulation in mammals (3, 4, 14, 46, 48). For example, both evaporative and non evaporative heat loss processes, such as panting in dogs (2) and tail skin vasodilatation in rats (30), are attenuated during systemic salt loading. In contrast, systemic salt loading facilitates heat-escape/cold-seeking behavior in rats (22, 33) and pigeons (5). Despite this physiological evidence demonstrating the modulation of thermoregulatory responses during osmotic stimulation, the underlying mechanism remains little known.

The preoptic area/anterior hypothalamus (PO/AH) contains abundant warm-sensitive neurons (35), which are involved in various autonomic and behavioral thermoregulatory processes (12, 13, 19, 31, 42, 43). It is generally considered that activation of the warm-sensitive neurons facilitates both autonomic and behavioral responses against the heat. Baker and Doris (3, 4) first reported that osmotic stimulation attenuates evaporative heat loss at the level of the hypothalamus. Nakashima et al. (34) showed that, in an in vitro slice of rat brain, the warm-sensitive neurons in the medial PO (MPO) lower the firing rate in a hyperosmotic medium. These results suggest that osmotic stimulation attenuates the central thermosensitivity to heat, resulting in the suppression of autonomic heat loss responses. However, this speculation would not be the case in heat-escape/cold-seeking behavior. Hori et al. (13) reported that activation of the warm-sensitive neurons in the MPO was also related to operant cold-seeking behavior in monkeys. However, if activation of the warm-sensitive neurons directly determined the behavioral response to the heat, the osmotic stimulus should have attenuated the operant heat-escape/cold-seeking behavior in our previous studies. Therefore, we surmised that a brain region other than the MPO would be involved in the facilitation of heat-escape/cold-seeking behavior during systemic salt loading.

Systemic salt loading exerts several actions through the brain mechanisms at the lamina terminalis (LT), including the subformical organ (SFO), the organum vasculosum lamina terminalis (OVLT), and the median preoptic nucleus (MnPO) (7, 24, 26, 28). For example, the LT modulates blood pressure, water and salt intakes, vasopressin secretion, and renal Na⁺ excretion during increases in extracellular osmolality and/or Na⁺ (11, 25, 27, 51). Intravenous infusion of hypertonic saline activates Fos expression in the nuclei in the LT, reflected by neural activity (36, 37). In particular, neurons in the MnPO also respond to brain temperature (49). Heat exposure in-
creases Fos expression in the MnPO (23), and the combination of heat and osmotic stimuli additively augments the Fos expression (38). Moreover, the MnPO receives the information regarding blood volume (1), which is another factor affecting thermoregulation (8, 14, 15). Therefore, in the present study, we hypothesized that the MnPO would be one of sites involved in the facilitation of heat-escape/cold-seeking behavior during osmotic stimulation. To test this hypothesis, we assessed operant heat-escape/cold-seeking behavior of rats, in which the MnPO lesion was induced with ibotenic acid. We assumed that the facilitation of behavior would be blunted in the MnPO-lesion animals.

METHODS

Male crj-Wistar rats (n = 32, 240–260 g; Charles River Japan, Osaka, Japan) were used in the present study. Rats were housed individually at an ambient temperature (T) of 23 ± 1°C in a 12:12-h light-dark cycle (lights on at 0700) and had free access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee, College of Health Science, Osaka University Graduate School of Medicine, Faculty of Human Sciences, Waseda University, and conformed to the United Kingdom Animals (Scientific Procedures) Act 1986.

Surgical preparations. Under general anesthesia induced by an intraperitoneal injection of pentobarbital sodium (Nembutal, 50 mg/kg; Dainippon, Osaka, Japan), the salivary ducts of the parotid and major sublingual and submaxillary glands were bilaterally ligated, and the glands were removed to minimize active evaporative heat loss. It has been reported that the submaxillary gland is the most important for evaporative heat loss in the heat (10). As reported in our previous study (32), rats without these major salivary glands could not control their body temperature during a heat exposure at 40°C. However, when behavioral process (i.e., heat-escape/cold-seeking behavior) is available, the rats can control body temperature in the environment. A radio transmitter (15 × 30 × 8 mm; Physiotel, Data Science, St. Paul, MN) was placed in the peritoneal cavity for the measurement of body temperature (Tcore). To produce a chemical lesion of the MnPO, the skull was incised, and a small hole was opened. A 30-gauge injection cannula (Unique Medical, Tokyo, Japan) was stereotaxically inserted into the brain through the hole. The coordinates used were 0.0 mm anteroposterior (AP), 6.5 mm dorsoventral (DV), and 0.0 mm mediolateral (ML) relative to the bregma (39). Ibotenic acid [α-amino-3-hydroxy-5-isoxazolyl acetic acid, 4.0 μg in 0.8 μl PBS (pH 7.4), n = 20; Tocris Cookson, Bristol, UK] or the vehicle (0.8 μl; n = 12) was injected at the rate of 40 nl/min (CMA 100; Carnegie Medicin, Stockholm, Sweden). After the injection, the syringe was left in position for 10 min to prevent back flow. Another stainless steel cannula for intracerebroventricular injection (∼0.9 mm AP, 3.5 mm DV, and 1.4 mm ML) was implanted in the right lateral ventricle and fixed to the skull with dental cement. To minimize postoperative discomfort, lidocaine jelly (Xylocaine jelly; AstraZeneca, London, UK) was applied to the area of the closed incision. Desalivated rats showed postsurgical polydipsia (usually drank more than 150% of the paired control rats), and successful desalivation was verified by postmortem examination.

After a 2-wk recovery from the surgery, we selected rats in the MnPO-lesion group, based on water intake after intracerebroventricular injection of ANG II. It was reported that central administration of ANG II stimulates drinking behavior (24, 28), and chemical lesions of the MnPO attenuate this response (18). ANG II (10 ng·1.0 μl1·100 g−1); [Sar1] ANG II, American Peptide, CA) was injected through the ventricular cannula, and water intake was measured for 30 min after the injection. Because it was difficult to conduct the MnPO lesion after the placement of the ventricular cannula, we just compared the water intake between the control and MnPO lesion rats. In the control rats, the volume of water intake after ANG II injection was 4.5 ± 0.2 ml/100 g. All of the MnPO-lesion rats drank less than this level; however, we used 15 out of 20 rats for the experiment, which drank water less than 60% of this average. Final judgment for successful lesion of the MnPO was made by histological analysis indicated later. Thus 12 out of 15 rats were selected as the MnPO-lesion group for analysis: their water intake was 0.1–2.7 ml/100 g (1.7 ± 0.4 ml/100 g in average). Water intake after the vehicle injection did not differ between the MnPO lesion (0.2 ± 0.1 ml/100 g) and control groups (0.3 ± 0.1 ml/100 g). For each rat in both groups, a silicone catheter (1.0 mm OD; Fuji Systems, Tokyo, Japan) was placed in the inferior vena cava through the femoral vein for blood sampling under general anesthesia, as described above. The other end was pulled out through the nape and plugged with a stainless steel rod. The catheter was flushed with heparinized saline (50 units/ml) daily to avoid clogging.

Experimental operant behavior system. The experimental system used for quantitative assessment of thermoregulatory behavior was reported previously (6). Briefly, the system comprised a Plexiglas box (30 × 10 × 30 cm) with many 1-cm holes in an environmental chamber (80 × 65 × 60 cm). The chamber was ventilated by either a warm (25–40°C) or cold (0–30°C) air-supply unit (CAU-210, Tabai Espec, Osaka, Japan), which was switched by computer-controlled valves. Five pairs of sensor-units located a freely moving rat to one of five 10 × 10 cm2 areas (area 1–5 from the left). Areas 4 and 5 were defined as the reward area: when a rat moved in the reward area during heat exposure at 35°C or 40°C, 0°C-air ventilated the chamber for 45 s (operant behavior). To receive another 0°C-air reward, the rat had to move out of the reward area and then back in again. Reentry to the reward area within 45 s of the previous reward did not trigger another reward. The T in the box and Tcore of a rat were continuously monitored with a thermocouple and by telemetry, respectively, and these data were stored on the same computer every 5 s. During the ventilation of 0°C air, the ambient temperature dropped to ~10°C. However, rats were not directly exposed to the 0°C air. Therefore, it is supposed that the cold air could not be a noxious stimulus (50), which modulates the cold-seeking behavior due to fear and pain. Moreover, Maruyama et al. (23) reported that this operant behavior did not increase Fos-IR cells in the supraoptic nucleus, amygdala, and lateral septum, which are observed during stress stimuli, such as immobilization and pain (44). At least 5 days after the venous catheter placement, a rat was put in the operant system set at 40°C for 2 h; this procedure was repeated 3 times with a 3-day interval (i.e., training session). All of the rats learned the operant behavior after this training session.

Experiment 1. Exposure to a 33°C environment without cold rewards. Twelve rats (the MnPO-lesion and control groups, n = 6 each) were used in this experiment. These rats did not learn the operant behavior but were placed in the operant system at 26°C with a similar time schedule to that of the training session. At 1000 on the experimental day, each rat was given subcutaneous injection (10 ml/kg) of either isotonic (154 mM NaCl) or hypertonic saline (2,500 mM NaCl) on its flank under local anesthesia with 0.5% lidocaine hydrochloride (0.5 ml; Xylocaine). The injection was conducted with the rat loosely wrapped with a towel on the investigator’s thigh. This procedure did not cause abnormal behavior, such as licking, biting, and scratching related to pain (21). Thirty minutes after the injection, the rat was put in the operant system set at 26°C for 60 min and then 33°C for another 90 min. The system was programmed, so as not to give cold air rewards. After a at least 1 wk of recovery, the rat repeated the same protocol with subcutaneous injection of the other tonicity of saline on the other side of the flank. The order of the two trials was randomized. Rats were deprived food and water during the experiment. After a 60-min exposure at 26°C, three rats in each group were also exposed to 35°C for 1 h, and then 40°C for another 1 h.

Experiment 2. Exposure to 35 and 40°C environments with cold rewards. For this experiment, the other 12 rats learning the operant behavior (the MnPO-lesion and control group, n = 6 each) were used.
Either isotonic or hypertonic saline was injected into the rat in the same manner as in experiment 1. The rat was placed in the operant system set at 26°C for the first 60 min, and then the system (i.e., loading air temperature) was set at 35°C for 1 h and then 40°C for another 1 h. During the baseline period at 26°C, the valves were switched off not to give 0°C air. Although rats were exposed to 35°C and 40°C air, 0°C air was available for 45 s by the operant behavior. The same protocol with the saline injection of the other tonicity was repeated for each rat with a 1-wk interval at least.

Blood sampling and measurements. In experiments 1 and 2, body weight was measured at three different time points: 1) before subcutaneous saline injection, 2) 30 min after the injection (just before a rat was put in the experimental system), and 3) at the end of the experiment. In experiment 2, blood samples (0.3 ml) were taken through the venous catheter at the same time points. Plasma osmolality (freezing-point depression; One-Ten osmometer, Fiske, Norwood, MA) and Na⁺ concentration (flame photometry; Corning, Medfield, OR) in the blood were determined. Hematocrit (Hct, microcentrifugation) and plasma protein concentration (PPC; refractometry, Atago, Tokyo, Japan) were measured to estimate the relative change in blood volume (29).

Histological analysis. At least 1 wk after experiment 1 or 2, all of the MnPO-lesion rats were given subcutaneous hypertonic-saline injection followed by a 2-h heat exposure at 35°C. Because both heat exposure and salt loading were reported to increase fos expression in the MnPO (23, 36–38), we assumed that the Fos response to the heat exposure and salt loading would be smaller in the lesion group of rats. In addition, 8 rats in the control group were divided into two subgroups (n = 4 each): one group received the isotonic saline injection followed by a 2-h heat exposure at 26°C, and the other received the hypertonic saline injection followed by a 2-h heat exposure at 35°C. Immediately after the exposure, each rat was killed by an intraperitoneal injection of a large dose of pentobarbital sodium (200 mg/kg) and was perfused transcardially with a fixative solution (300 ml, 4% paraformaldehyde in PBS). The brain was quickly removed and stored at 4°C in the fixative solution for 6 h and then in 25% sucrose in PBS for another 48 h. Next, 40-μm coronal sections were prepared. The sections were reacted with 0.3% hydrogen peroxide in PBS with 0.3% Triton X-100 for 30 min and incubated with rabbit primary anti-Fos polyclonal IgG (1:4,000 dilution; Santa Cruz, CA) for 12 h. After rinsing with PBS, the sections were incubated again in biotinylated donkey anti-rabbit IgG (1:400 dilution; Vector Laboratories, Burlingame, CA) for 90 min, avidin-biotin complex (1:400 dilution; Vector Elite Kit, Burlingame, CA) for another 90 min, and then 0.02% diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO) in PBS. The sections were mounted on gelatin-coated glass slides, and counterstained with 0.02% thionin solution (Nissl stain), and then coverslipped. After digital images of the sections were captured (model E600; Nikon, Tokyo, Japan and model IK-TU; 42, Toshiba, Japan), Fos-IR cells in the MnPO, OVLT, SFO, and MPO were counted. In addition, the extension of the lesion was assessed by counting large cells (neural body) in the rostral, middle, and caudal part of the MnPO. We defined the large cell, of which diameter was larger than 10 μm. If the large cells resided in more than 50% of the control, the rat was excluded from the analysis. The mapping was conducted, based on the atlas of Paxinos and Watson (39). Fos-IR cells in the MnPO were counted in three consecutive sections around the injection level. Those in the MPO were also counted in the rostral, middle, and caudal parts. Counting was conducted by a person who was not informed of the details of the experiment.

Statistical analysis. Differences among means in water intake, Tcore, counts of the operant behavior, Ta, body weight, blood parameters, and counts of Fos-IR cells were assessed by ANOVA with repeated measurements or one-way ANOVA. A post hoc test to identify a significant difference at a specific time point was performed by the Newman-Keuls procedure. A null hypothesis was rejected at the level of P < 0.05. All values were presented as means ± SE.

RESULTS

Experiment 1. Exposure to a 33°C environment without cold-air rewards. Figure 1 shows Tcore during the 33°C exposure (A) and the change from the baseline (the average for the latter 30 min during 26°C exposure, ∆Tcore, B and C) in the control and MnPO-lesion groups with the isotonic- and hypertonic-saline injections. In the isotonic-saline trial, the baseline Tcore was similar in the two groups (37.5 ± 0.1°C). Although Tcore in the control group remained at the level throughout the 33°C exposure, Tcore in the MnPO-lesion group became higher (P < 0.05) than that in the control group at 45–90 min (Fig. 1A; 38.0 ± 0.1°C and 37.5 ± 0.1°C at 90 min, respectively). The baseline Tcore in the hypertonic-saline trial was also similar in both groups (36.3 ± 0.1°C), but lower (P < 0.05) than the values in the isotonic-saline trial. In contrast to the isotonic-saline trial, Tcore in the hypertonic-saline trial was lower (P <
0.05) in the MnPO-lesion group than the control group at 50–90 min.

In the control group ΔT\text{core} was greater (\(P < 0.05\)) in the hypertonic- than the isotonic-saline trial at 30–90 min (by 1.5 ± 0.1°C at 90 min; Fig. 1B). However, ΔT\text{core} was not different between the two trials in the MnPO-lesion group throughout the 33°C exposure (Fig. 1C).

There was no difference in T\text{core} between the MnPO-lesion and control rats (\(n = 3\) each) exposed to 35°C for 1 h and 40°C heat for another 1 h without operant behavior. In both groups, T\text{core} similarly elevated during a 1-h exposure at 35°C (38.5 ± 0.1°C and 38.4 ± 0.1°C at the end in the MnPO-lesion and control groups, respectively). Under the heat at 40°C, T\text{core} in both the control and MnPO-lesion groups surpassed 40.0°C; thus we stopped the experiment at 25 ± 2 min and 24 ± 2 min after the onset, respectively.

**Experiment 2. Exposure to 35 and 40°C environments with cold-air rewards.** Figure 2 illustrates examples of the operant heat-escape/cold-seeking behavior with T\text{core} and T\text{a} in the isotonic- (A and C) and hypertonic-saline (B and D) trials in the control (A and B) and MnPO-lesion (C and D) groups. No rat moved much in the behavior box during the latter 30 min of 26°C exposure; however, the animals periodically went in and out of the reward area during the 35°C and 40°C exposure. In all of the examples, operant behavior increased with an increase in the loading temperature. In the control rat, the operant behavior seemed to be augmented in the hypertonic-saline trial (Fig. 2A and B), compared with that in the isotonic-saline trial. However, this response appeared to be less in the MnPO-lesion rats (Fig. 2, C and D).

Figure 3 shows the average T\text{core} and T\text{a} and counts of the operant behavior in the last 30 min during 26°C exposure (baseline) and each 30 min during the 35°C and 40°C exposure in the control and MnPO-lesion groups. Similar to **experiment 1**, the baseline T\text{core} in the control group was lower (\(P < 0.05\)) in the hypertonic than the isotonic-saline trial (Fig. 3A). T\text{core} in each trial did not change from the baseline in the heat. The baseline counts during the 26°C exposure (i.e., nonspecific movement) was similar in both trials (2 ± 1 and 4 ± 1 in the isotonic- and hypertonic-saline trials, respectively; Fig. 3B). Operant behavior increased (\(P < 0.05\)) with an increase in the loading temperature in each trial; however, the counts were greater (\(P < 0.05\)) in the hypertonic- than the isotonic-saline trial (17 ± 2 and 10 ± 1 at 35°C, and 24 ± 2 and 18 ± 1 at 40°C, respectively). The average T\text{a} in the hypertonic- and isotonic-saline trials was 29.5 ± 0.9°C and 32.5 ± 0.2°C at 30–60 min (in 35°C heat), and 31.2 ± 0.5°C and 33.6 ± 0.6°C at 90–120 min (in 40°C heat), respectively (Fig. 3C).

In the MnPO-lesion group, T\text{core} in each trial increased (\(P < 0.05\)) from the baseline during the 35°C and 40°C exposure. However, T\text{core} remained lower (\(P < 0.05\)) in the hypertonic-than the isotonic-saline trial throughout the experiment (\(37.4 ± 0.1°C\) and 37.7 ± 0.2°C at 120 min, respectively; Fig. 3D). The counts of operant behavior were similar in the two trials (2 ± 1 and 3 ± 1 in the baseline, 8 ± 1 and 10 ± 1 at 35°C, and 16 ± 1 and 17 ± 1 at 40°C in the isotonic- and hypertonic-saline trials, respectively; Fig. 3E). The average T\text{a} was 32.1 ± 0.5°C and 31.4 ± 0.5°C at 35°C, and 33.8 ± 0.7°C and 33.8 ± 0.3°C at 40°C in the hypertonic- and isotonic-saline trial, respectively; Fig. 3F).

![Example of operant behavior in experiment 2](AJP-Regul Integr Comp Physiol • VOL 292 • JANUARY 2007 • www.ajpregu.org)
Tcore and the counts of the operant behavior in the isotonic-saline trial were not different between the control and MnPO-lesion groups. Although, in the hypertonic-saline trial, Tcore was also similar in the two groups, the counts of operant behavior were smaller in the MnPO-lesion group.

Changes in body weight and blood parameters. In experiments 1 and 2, body weight before subcutaneous saline injection was lower (P < 0.05) in the MnPO-lesion than the control group, although they were the same age in weeks (330 ± 8 g and 400 ± 8 g in experiment 1 and 343 ± 7 and 401 ± 9 in experiment 2, respectively). In experiment 2, percent reduction of body weight was greater (P < 0.05) in the hypertonic- than the isotonic-saline trial in the two groups (5 ± 1% and 1 ± 1% in the control, and 4 ± 1% and 1 ± 1% in the MnPO-lesion group, respectively). Baseline osmolality and Na⁺ concentration in the plasma were greater (P < 0.05) in the MnPO-lesion than the control group (Table 1). The two parameters in the isotonic-saline trial remained unchanged in the control group. In the MnPO-lesion group, plasma osmolality decreased to the level in the control group (301 ± 2 mosmol/kg H₂O) 30 min after the isotonic-saline injection and returned to the preinjection level at the end of experiment. Osmolality and Na⁺ concentration in the plasma in the hypertonic-saline trial increased (P < 0.05) 30 min after the injection in both groups.

Table 1. Plasma osmolality and sodium concentration in experiment 2

<table>
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<tr>
<th>Group</th>
<th>Trial</th>
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<td>302±1</td>
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Values are expressed as means ± SE (n = 6). Data were obtained before (baseline), 30 min after a subcutaneous saline injection, and at the end of experiment.

*Significant difference between the isotonic- and hypertonic-saline trials in each group, P < 0.05. †Significant difference from the baseline value in each trial, P < 0.05. ‡Significant difference between the control and MnPO-lesion groups in the isotonic- and hypertonic-saline trials, P < 0.05.
(321 ± 2 mOsm/kg·H₂O) and 331 ± 2 mOsm/kg·H₂O) in osmolality, and 156 ± 1 mmol/l and 160 ± 2 mmol/l in Na⁺ concentration in the control and MnPO-lesion group, respectively), and remained unchanged until the end of the experiment. Estimated from changes in Hct and PPC, blood volume in each group increased (P < 0.05) 30 min after the isotonic- and hypertonic-saline injections (5 ± 2% and 6 ± 2% in the control group, and 5 ± 2% and 4 ± 1% in the MnPO-lesion group, respectively). At the end of the experiment, blood volume in the isotonic-saline trial returned to the baseline level (1 ± 2% and 2 ± 1% in the control and MnPO-lesion groups, respectively); however, that in the hypertonic-saline trial remained increased (6 ± 3% and 5 ± 1% in the control and MnPO-lesion groups, respectively).

**Histological analysis.** Photo images of the brain sections in the control and MnPO-lesion rats are shown in Fig. 4. Counts of Fos-IR cells in the MnPO, OVLT, SFO, and MPO were summarized in Fig. 5. In the MnPO area, there are more Fos-IR cells in the control rats (Figs. 4A, a and c and 5A) but less in the MnPO-lesion rats (Fig. 4A, b and d). The glial cells (i.e., small cells) substituted for normal neurons in and around the MnPO area in the rats with an MnPO lesion. In the control group, counts of large cells (>10 µm in diameter) representing neurons were 210 ± 10, 329 ± 22, and 282 ± 25 in the rostral, middle, and caudal parts of the MnPO, respectively. In the MnPO-lesion group, the counts were 35 ± 8, 24 ± 8, 29 ± 10, respectively. In the lesion group, enlargement of the third ventricle and partial destruction of the anterior commissure were observed. However, we could find no apparent deformity of other neural areas and structures. In the control group with the hypertonic-saline injection and heat exposure, Fos-IR cells increased (P < 0.05; Fig. 5A). However, counts of Fos-IR cells were less (P < 0.05) in the MnPO-lesion rats with the same stimuli and were similar to those in the control group with the isotonic-saline injection and 26°C exposure. These histological differences in the MnPO area between the MnPO-lesion and control groups were correlated to the difference in water intake after the intraventricular ANG II injection. In the OVLT (Figs. 4B, a and b and 5B), SFO (Figs. 4C, a and b and 5C), and MPO (Figs. 4D, a and b and 5D), counts of Fos-IR cells in both groups were greater (P < 0.05) after the hypertonic-saline injection and 35°C heat; however, there were no differences in the counts between the two groups.

**DISCUSSION**

In the present study, we estimated the role of the MnPO in the facilitation of operant heat-escape/cold-seeking behavior after the hypertonic-saline injection. After the hypertonic-saline injection, rats in the control group could not maintain their body temperature at Tₐ of 33°C. When the operant behavior was available, they could regulate T core during 35°C and 40°C heat by increasing the operant behavior. On the contrary, these responses were abolished in the MnPO-lesion rats.
Experiment 1. Exposure to a 33°C environment without cold-air rewards. Previous studies have indicated an involvement of the MnPO in autonomic heat loss processes. Whyte and Johnson (52) showed that after the formation of lesions of the anteroventral third ventricle regions (AV3V), including the ventral MnPO and OVLT, salivary spreading during a 37°C exposure was attenuated in rats, resulting in an augmented rise in T_core. Moreover, it was reported that the MnPO has multisynaptic efferent connections with autonomic preganglionic neurons controlling the salivary glands (16, 17) and tail vasculature (45) in rats by way of several preautonomic regions, including paraventricular nucleus, rostral ventrolateral or ventromedial medulla, and periaqueductal gray matter. In the present study, we used desalivated rats to minimize the evaporative process. The rats could not maintain their T_core during the 35°C and 40°C exposures, regardless of the MnPO lesion. Even during exposure at 33°C, the reported upper limit of the thermoneutral range [22–34°C; (9)], T_core in the MnPO-lesion group increased by 0.5°C in the isotonic-saline trial (Fig. 1B). This result may also indicate an involvement of the MnPO in dry heat loss processes.

The baseline T_core at the T_a of 26°C was lower in the hypertonic- than the isotonic-saline trial in both the control and MnPO-lesion groups (Fig. 1A). We previously reported that, after the hypertonic-saline injection, T_core decreased with metabolic rate, and an increase in metabolic rate during a cold exposure was attenuated (21). Therefore, it seems that T_core is maintained lower in thermoneutral and cold environments during the osmotic stimulus, by the suppression of metabolic heat production. However, the present study indicates that the MnPO is not involved in the reduction of T_core after the hypertonic-saline injection. ΔT_core (i.e., the change from the baseline) during the 33°C exposure was greater in the hypertonic- than the isotonic-saline trial in the control group (Fig. 1B), but not in the MnPO-lesion group (Fig. 1C). It is well known that an increase in osmolality or Na⁺ in the plasma attenuates both evaporative and dry heat loss processes (2–4, 30, 46, 48). Because we used desalivated rats in this study, the greater ΔT_core in the hypertonic-saline trial may be reflected by the suppression of dry heat loss processes. T_core increased in the MnPO-lesion group during the 33°C exposure in the isotonic-saline trial, although the saline injection decreased plasma osmolality 30 min after the injection. Moreover, the increase in T_core in the hypertonic-saline trial was largely attenuated in the MnPO-lesion group, despite greater increase in plasma osmolality. Thus the MnPO itself may control dry heat loss in part. Moreover, the MnPO responds to osmotic stimulation and attenuates dry heat loss processes.

Experiment 2. Exposure to 35°C and 40°C environments with cold-air rewards. Rats in both the control and the MnPO-lesion groups maintained their T_core during the 35 and 40°C exposures when the operant behavior was readily available (Fig. 3). Moreover, the operant behavior increased with the loading temperature. These results suggest that skin temperature is a major trigger for the operant heat-escape/cold-seeking behavior in both groups, although T_core is considered to be another factor (13, 40, 42, 43).

T_core and counts of behavior during the 35°C and 40°C exposures when the operant behavior was readily available (Fig. 3). Moreover, the operant behavior increased with the loading temperature. These results suggest that skin temperature is a major trigger for the operant heat-escape/cold-seeking behavior in both groups, although T_core is considered to be another factor (13, 40, 42, 43).
In the present study, rats controlled Ta around 29 – 34°C on experiment 1 to strengthen the hormonal responses to osmotic stimulus. To level. These results indicate that thermal stimulation may also influence by the desalivation in our preliminary finding. Thus we suppose the role of the MnPO in the operant heat-escape/cold-seeking behavior would be small.

As we previously reported (32), the hypertonic-saline injection facilitated the operant behavior during the 35°C and 40°C exposure in the control group (Fig. 3B). Because of the increase in the operant behavior, the average Ta in the hypertonic-saline trial became lower than that in the isotonic-saline trial. This response would be important in thermoregulation during osmotic stimulation. When the operant behavior was not available, Tcore gradually increased even during the 33°C exposure (experiment 1, Fig. 1). The rats maintained Tcore during the 35°C and 40°C exposure by increasing the operant behavior (experiment 2; Figs. 2 and 3). However, in the MnPO-lesion group, there was no effect of the hypertonic-saline injection on operant behavior (Fig. 3E). These results suggest that the MnPO is involved in the facilitation of operant heat-escape/cold-seeking behavior during osmotic stimulation. Because Tcore in the control group remained unchanged during operant behavior in both trials, osmotic stimulation may strengthen the thermal responsiveness to the increase in environmental temperature.

A possible explanation for the difference in behavior in the hypertonic-saline trial between the control and MnPO-lesion groups may be just a result of the difference in autonomic heat loss response. That is, greater attenuation in dry heat loss in the hypertonic-saline trial in the control group (Fig. 1B) augmented behavioral response to maintain body temperature, and less influence of hyperosmolality in the MnPO-lesion group on dry heat loss resulted in unchanged behavior (Fig. 1C). However, in the hypertonic-saline trial in the control group, the behavior was augmented and Tcore was maintained at lower level than the control. Moreover, different from the control group, Tcore increased from the baseline level without a change in the behavior in the isotonic-saline trial in the MnPO-lesion group. These results may indicate that the efficiency of dry heat loss response did not determine the behavior, as we have suggested in previous studies (21, 22, 32). Thus we suppose that the behavior responds to thermal inputs themselves, and the osmotic inputs to the MnPO modulate this response. Although we used desalivated rats, Rodland and Hainsworth (41) reported that, in normal rats, the role of salivation for total evaporative water loss was 0% at Ta of 30°C and 60% at 40°C. In the present study, rats controlled Ta around 29 – 34°C on average. Learning of the operant behavior was not much influenced by the desalivation in our preliminary finding. Thus evaporative heat loss may not affect the behavior much, either, and the behavior would be activated independently of autonomic heat loss responses.

Keil et al. (20) reported that, in rabbits, plasma vasopressin level during hypertonic-saline infusion was augmented by hypothalamic warming and was attenuated by hypothalamic cooling. In contrast, isotonic control had no effect of vasopressin level during the hypothalamic warming and cooling. A similar but weak effect was also observed in the corticosterone level. These results indicate that thermal stimulation may also strengthen the hormonal responses to osmotic stimulus. Together, with the results in the present study, there may be bidirectional connections between the mechanisms involved in thermo- and osmoregulations.

Histological analysis indicates that the increase in Fos-IR cells after the hypertonic-saline injection with 35°C heat was attenuated in the MnPO-lesion rats. However, in other organs in the LT, such as the OVLT and SFO, the counts of Fos-IR cells were not different between the control and MnPO-lesion groups. Moreover, 80 – 90% of neurons in the MnPO were successfully destroyed in the lesion group, although we estimated it only by counting large-size cells (>10 μm) in the area. Oldfield et al. (36, 37) reported that osmotic stress increased Fos expression in these nuclei in the LT, which is involved in body fluid regulation and blood pressure control (11, 25, 27, 51). The Fos expression in the MnPO also responds to the heat (23, 49). In the present study, we did not estimate separately the influence of the osmotic and heat stresses on the Fos expression. However, the results may suggest that the neural responses to the osmotic and/or heat stresses were similar in both the control and MnPO-lesion rats. Moreover, among the organs in the LT, the MnPO may play an important role in the modulation of the behavioral and/or autonomic thermoregulation in the heat by osmotic stimulus.

There was no difference in the counts of Fos-IR cells in the MnPO between the MnPO-lesion and sham groups in the heat with osmotic stimulus, although the counts in each group increased from that in the control condition (i.e., at 26°C with isotonic-saline injection). This result may show that the activation of the MnPO due to the heat and/or osmotic stresses had little influence on that of the MPO. In an in vitro slice of rat brain, osmotic stimulation suppresses the activity of warm-sensitive neurons in the MnPO (34). All the Fos-IR cells in the MPO would not be the warm-sensitive neurons. In addition, we did not test whether the Fos-IR cells in the heat decrease during the osmotic stress. However, the results may indicate that the change in the behavioral and/or autonomic responses during the osmotic stress did not reflect neural activities in the MPO, estimated by Fos expression. Thus we speculate that the MnPO does not modulate behavioral and/or autonomic thermoregulation just by transferring osmotic signal to the MPO but plays a specific role in the thermoregulatory modulation independent of the MPO.

Numerous studies have shown that several autonomic heat loss responses to the heat are suppressed during dehydration or systemic salt loading by the factors of hyperosmolality and/or hypernatremia (2 – 4, 30, 46, 48) and hypovolemia (8, 14, 15). It seems that most investigators explain this phenomenon as an upward shift of the set point Tcore for thermoregulation. Indeed, the threshold Tcore for evaporative and nonevaporative heat loss processes was reported to elevate during dehydration or salt loading (14, 30). The report by Nakashima et al. (34) that the activity of warm-sensitive neurons in the MPO was suppressed by osmotic stimulation tends to support this concept. On the other hand, we have reported that salt loading decreases Tcore, suppresses metabolic heat production to a cold stimulus (21), and increases heat-escape/cold-seeking behavior in the heat (32). Therefore, the concept of an upward shift of the set point Tcore could not explain all of the thermoregulatory modulation during osmotic stimulation.

The study showed the importance of the MnPO in behavioral thermoregulation; however, the neural mechanism involved in...
the behavior remains unclear and how the MnPO affects it, also. Maruyama et al. (23) reported, using Fos immunostaining, an involvement of the MnPO, the dorsomedial hypothalamus, and the parastrial nucleus in the same operant behavior as we assessed in the present study. The dorsomedial hypothalamus receives neurons from the MnPO and the parastrial nucleus (47). In addition, the dorsomedial hypothalamus has telencephalic inputs from the ventral subiculum and the prefrontal cortex, which is related to behavior and memory. The dorsomedial hypothalamus is also known to be associated with heating-induced grooming in rats (53). Therefore, it may be supposed that the dorsomedial hypothalamus plays a key role in behavioral thermoregulation, and the MnPO has an influence on it.

Autonomic responses to the heat are suppressed during dehydration, that is, water depletion and increase in solute concentration in the body fluid compartments. However, the present study showed that there would be a specific mechanism activating thermal sensation and/or discomfort to the heat, that is, an increase in behavioral response, which prevents an increase in body temperature. Precise knowledge about such a mechanism might be applied to the prevention of heat stroke, for which dehydration is one possible factor.

In conclusion, the MnPO may play an important role in the facilitation of heat-escape/cold-seeking behavior during systemic salt loading in rats. In addition, the present data also indicate a possible role of the MnPO in the attenuation of dry heat loss response during osmotic stimulation. The MnPO may be a key area integrating the regulatory mechanisms of body temperature and fluid balance.

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REFERENCES


