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Systemic salt loading decreases body temperature and increases heat-escape/cold-seeking behaviour via the central AT_1 and V_1 receptors in rats

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Salt loading decreases body core temperature (T_{core}) at neutral ambient temperature (26 °C) and increases heat-escape/cold-seeking behaviour in desalivated rats. In this study, we tested the hypothesis that brain angiotensin II (AII) and arginine vasopressin (AVP) are associated with these responses. Surgically desalivated rats ($n = 28$) were administered an injection (s.c., 10 ml kg^{-1}) of either normal saline (154 mM, NS) or hypertonic saline (2500 mM, HS) following an intracerebroventricular injection (10 μl kg^{-1}) of an AII AT_1-receptor antagonist (candesartan, 5 μg μl^{-1}), an AVP V_1-receptor antagonist (((β-mercapto-β, β-cyclopenta-methylene propionyl^1, O-Me-Tyr^2, Arg^8)-vasopressin, 0.5 μg μl^{-1}), or normal saline (154 mM). Each rat was placed in a behaviour box, first at 26 °C for 1 h to allow the measurement of baseline T_{core} and movement. The ambient temperature was then elevated to 40 °C for the next 2 h, during which time the rat was able to trigger a 0 °C air reward for 30 s by moving into a specific area of the box (operant behaviour). The s.c. HS significantly decreased baseline T_{core} at 26 °C (36.5 ± 0.1 °C) and increased counts of operant behaviour at 40 °C (57 ± 3) compared with results obtained following s.c. NS injection (37.4 ± 0.1 °C and 42 ± 1, respectively). These responses to s.c. HS were inhibited by the intracerebroventricular injection of AT_1 (37.3 ± 0.1 °C and 43 ± 2, respectively; $P < 0.05$) and V_1 antagonists (37.2 ± 0.2 °C and 42 ± 2, respectively; $P < 0.05$), although administration of both antagonists with s.c. NS had no effect. These results suggest that brain AII and AVP are involved in the decrease in T_{core} observed at neutral ambient temperature and the increase in heat-escape/cold-seeking behaviour in response to osmotic stimulation, via the central AT_1 and V_1 receptors, respectively

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It is well known that autonomic heat loss processes such as panting, sweating, salivary secretion and skin vasodilatation are attenuated during dehydration or salt loading due to the accompanying plasma hyperosmolality and/or hypovolaemia (Baker & Doris, 1982; Horowitz & Nadel, 1984; Baker & Dawson, 1985; Horowitz & Meiri, 1985; Thornton & Proppe, 1988; Nakajima *et al.* 1998). These alterations in autonomic heat loss responses are beneficial for body fluid conservation during dehydration, but they inevitably reduce heat tolerance. Our previous study, however, clarified that heat-escape/cold-seeking behaviour in rats increased after s.c. hypertonic saline injection (Nagashima *et al.* 2001). This result (Nagashima *et al.* 2001) suggests that the increase in the behaviour effectively compensates for the reduced autonomic responses, thus preventing hyperthermia, when a cooler environment is available. However, little is known about the mechanism underlying this phenomenon.

An increase in plasma osmolality during dehydration and/or salt loading elevates angiotensin II (AII) and arginine vasopressin (AVP) concentrations in both the plasma and cerebrospinal fluid (CSF; Thrasher *et al.* 1980; Simon-Oppermann *et al.* 1983, 1986; Szczepanska-Sadowska *et al.* 1983, 1984*a*, *b*; Doris & Bell, 1984; Eriksson *et al.* 1987; Jolkkonen *et al.* 1988). These two hormones have binding sites throughout the brain, and AT_1 receptors for AII and V_1 receptors for AVP are abundant (Phillips *et al.* 1988; Gerstberger & Fahrenholz, 1989; McKinley *et al.* 1990). Many studies have established that brain AII induces various autonomic and behavioural responses in body fluid regulation, such as AVP secretion and water intake via the central AT_1 receptors (Hogarty *et al.* 1994; Kregel *et al.* 1994; McKinley *et al.* 1994; Rohmeiss *et al.* 1995; Mathai *et al.* 2000). In addition, central AVP has also been shown to facilitate drinking behaviour (Szczepanska-Sadowska *et al.* 1982, 1983, 1984*a*, *b*). Moreover, a central injection of AVP also activates behavioural responses such as locomotion and grooming (Balaban *et al.* 1988; Diamant & De Wied, 1993; Lumley *et al.* 2001).

Several lines of evidence suggest that brain AII and AVP are also involved in body temperature regulation. In febrile rats, brain AVP seems to work as an endogenous

antipyretic substance, and the ventral septal area is considered to be a target region (Cooper *et al.* 1986). Intracerebroventricular (I.C.V.) injection of AII or AVP has been shown to induce hypothermia in rats (Shido & Nagasaka, 1985; Naylor *et al.* 1986). Kiyohara *et al.* (1984) demonstrated that a microinjection of AII into the medial preoptic area (MPO) in rats activated warm-sensitive neurons with a decrease in core temperature (T_{core}). Therefore, we believe that brain AII and/or AVP mediate various autonomic and behavioural responses in thermoregulation in response to osmotic stimulation.

In the study presented here, we evaluated the effects of I.C.V. injection of AT_1- and V_1-receptor antagonists on heat-escape/cold-seeking behaviour after S.C. hypertonic saline injection in desalivated rats, using an operant system (Chen *et al.* 1998). We hypothesized that both antagonists would suppress the increase in the thermoregulatory behaviour observed during salt loading.

METHODS

Male crj-Wistar rats (260–280 g; Charles River Japan, Osaka, Japan) were used in this study. The rats were housed individually at a room temperature of 23 °C in a 12:12 h light:dark cycle (lights on at 08.00 h) and had free access to food and water. All experimental procedures were approved by the Animal Committee of Osaka University Faculty of Medicine.

Surgical preparations

Under general anaesthesia induced by I.P. sodium pentobarbitone (pentobarbital; 50 mg (kg body wt)$^{-1}$), each rat underwent the following surgery: (1) intra-abdominal placement of a radio transmitter (15 × 30 × 8 mm; Physiotel, Data Science, St Paul, USA) for measurement of T_{core}; (2) ligation of the salivary ducts of the bilateral parotid and major sublingual and submaxillary glands following a previously reported procedure (Lipton & Marrotto, 1969) to minimize active evaporation, and (3) stereotaxic implantation of a stainless steel cannula (length: 5.0 mm, o.d. 0.7 mm) in the right lateral ventricle (0.92 mm posterior to the bregma, 1.4 mm lateral from the midline and 3.5 mm below the skull surface) for I.C.V. injection. The cannula was fixed to the skull with dental cement. To minimize post-surgical discomfort, lignocaine (lidocaine) jelly (Xylocaine jelly, AstraZeneca, London, UK) was applied to the area of the closed incisions. Successful desalivation was verified by post-surgical polydipsia. At least 2 weeks after the surgery, a silicone catheter (1.0 mm o.d.; Fuji Systems, Tokyo, Japan) for blood sampling was placed in the inferior vena cava through the right femoral vein, again under I.P. sodium pentobarbital (50 mg kg^{-1})-induced general anaesthesia. The other end of the catheter was exited at the nape and plugged with a stainless steel rod. To avoid clogging, the catheter was flushed with heparinized saline (50 units ml^{-1}) every day.

Operant behavioural system

The experimental system used for quantitative assessment of the thermoregulatory behaviour has been reported previously (Chen *et al.* 1998) and is shown in Fig. 1. Briefly, the system comprised a Plexiglas box (dimensions 50 × 10 × 30 cm) with many holes (1 cm diameter) that was placed in an environmental chamber (dimensions 80 × 65 × 60 cm; Fig. 1*A*). The chamber was well ventilated with air from either a warm (operative range, 25–40 °C) or a cold (0–30 °C) air-supply unit (CAU-210, Tabai Espec, Osaka, Japan), which could be switched on or off by computer-controlled valves. Five pairs of photosensor units consisting of a light-emitting diode (LED) and a photoelectric cell were located at the position of a freely moving rat in the box to one of five 10 × 10 cm areas (Fig. 1*B*). The computer received an input from the photosensors and gave an output (on/off) to the switching valves. The ambient temperature (T_a) in the box and T_{core} of a rat were monitored with a thermocouple and by telemetry, respectively, and those data were stored on the same computer every 5 s.

The temperature of the two air-supply units was set at 40 and 0°C, and air at 40 °C ventilated the chamber. The 40 °C air was switched to 0 °C air for 30 s when a rat moved into areas four or five (reward areas, Fig. 1*B*) in the box. To get another 0°C air reward, the rat

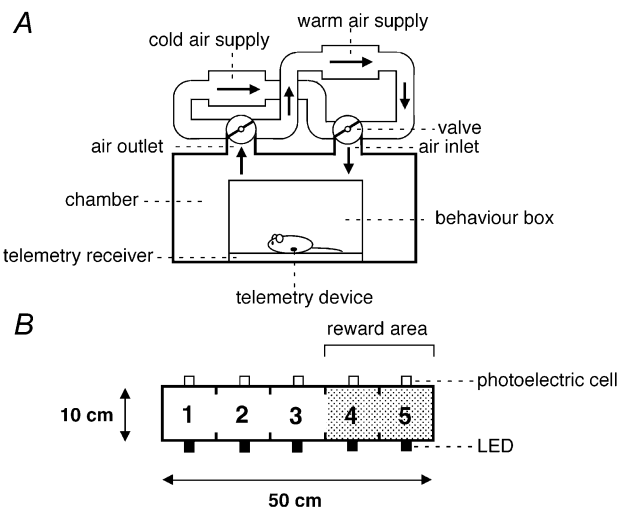

Figure 1. The experimental system

A, a Plexiglas box was positioned in a chamber, which was connected to warm (25–40 °C) and cold (0–30 °C) air-supply units. The ventilating air was switched on or off by computer-controlled valves. *B*, a top view of the behaviour box. The position of the rat in the box was determined by five pairs of light-emitting diodes (LED) and photoelectric cells, which divided the box into five 10 × 10 cm square areas in a single plane (1–5). The hatched area (4 or 5) was defined as the reward area.

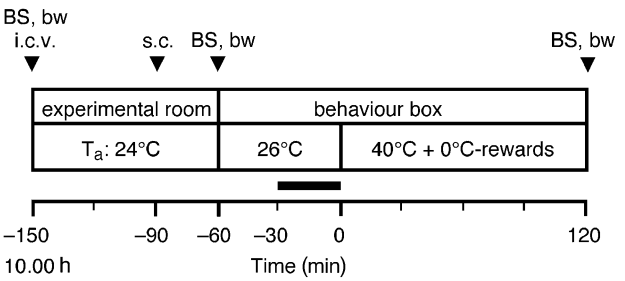

Figure 2. Schedule of the experiment

The horizontal bar shows the period of the baseline measurement. After a 1 h 26 °C period, a 2 h operant program (40 °C air ventilation with 0 °C air rewards) was conducted. BS, blood sampling; bw, body weight measurement; I.C.V., intracerebroventricular injection; S.C., subcutaneous injection; T_a, ambient temperature.

had to move out of the reward area and move back there again after the 0 °C air reward had finished (operant behaviour; i.e. a re-entry into the reward area within the duration of the 30 s period of 0 °C air reward did not trigger another reward and did not count as operant behaviour). There was no gradient of the air temperature in the five areas of the box during the heat and cold exposure. At least 1 week after the last surgery, each rat had three 2 h training sessions with at least a 2 day interval between them. All the rats were accustomed to the experimental system and easily learned the procedure of operant behaviour (Chen *et al.* 1998).

Subcutaneous and I.C.V. injections

Twenty eight rats were divided into two groups (n = 14 in each group): the animals in one group were given a S.C. injection (10 ml kg^{-1}) of hypertonic saline (HS, 2 500 mM NaCl) and those in the other group were given a S.C. injection of normal saline (NS, 154 mM NaCl). Each group was then subjected to three different experimental trials of I.C.V. injection (10 μl kg^{-1}) of the following substances: (1) normal saline (154 mM); (2) an AII AT_1-receptor antagonist (candesartan, 5 μg μl^{-1} in saline; Takeda, Osaka, Japan), and (3) an AVP V_1-receptor antagonist (((β-mercapto-β, β-cyclopenta-methylene propionyl^1, O-Me-Tyr^2, Arg^8)-vasopressin, 0.5 μg μl^{-1} in saline; Sigma, St Louis, MO, USA). Each rat had two of these trials: saline and AT_1 antagonist or saline and V_1 antagonist in random order.

Experimental protocol

Figure 2 shows the experiment schedule. The first experiment was conducted at least 3 days after the last training session. The I.C.V. injection (saline, AT_1 antagonist or V_1 antagonist) was first made through the ventricular cannula over a 60 s period (−150 min). One hour after the I.C.V. injection (−90 min), the S.C. injection (HS or NS) was performed in the lower back under local anaesthesia with 0.5 ml of 0.5 % lidocaine hydrochloride (Xylocaine). Thirty minutes after the S.C. injection (−60 min), the rat was put in the box. Both air-supply units were set at 26 °C for 1 h. T_{core} and movement became stable in the last half of the period, and these were defined as the baseline values. After 1 h at 26 °C, a 2 h operant behaviour programme, similar to the training session, was started by changing the air temperature to 40 °C and the reward to 0 °C. Animals were deprived of food and water during the experiment.

The second experiment was carried out on the same rats after an interval of at least 1 week. In it the rats were given the I.C.V. injection of the other fluid with the same S.C. injection on the other side of the back (e.g. the I.C.V. saline injection was conducted after the S.C. HS injection if the rat had the I.C.V. injection of AT_1 antagonist after the S.C. HS injection in the first experiment). The order of the two different I.C.V. injections was randomized. All other procedures were the same as in the first experiment.

Body weight measurement and blood sampling

Body weight measurement and blood sampling were repeated three times (Fig. 2): (1) before the I.C.V. injection (−150 min); (2) 30 min after the S.C. injection (−90 min) and (3) at the end of experiment (120 min). Haematocrit (Hct, microcentrifugation), plasma protein concentration (PPC, refractometry; Atago, Tokyo, Japan) and plasma osmolality ($osmol_{pl}$, freezing-point depression; One-Ten osmometer, Fiske, USA) were determined for all of the blood samples.

After the second experiment was completed, each rat was killed by I.P. injection of a large dose of sodium pentobarbital (200 mg kg^{-1}). Proper positioning of the ventricular cannula in

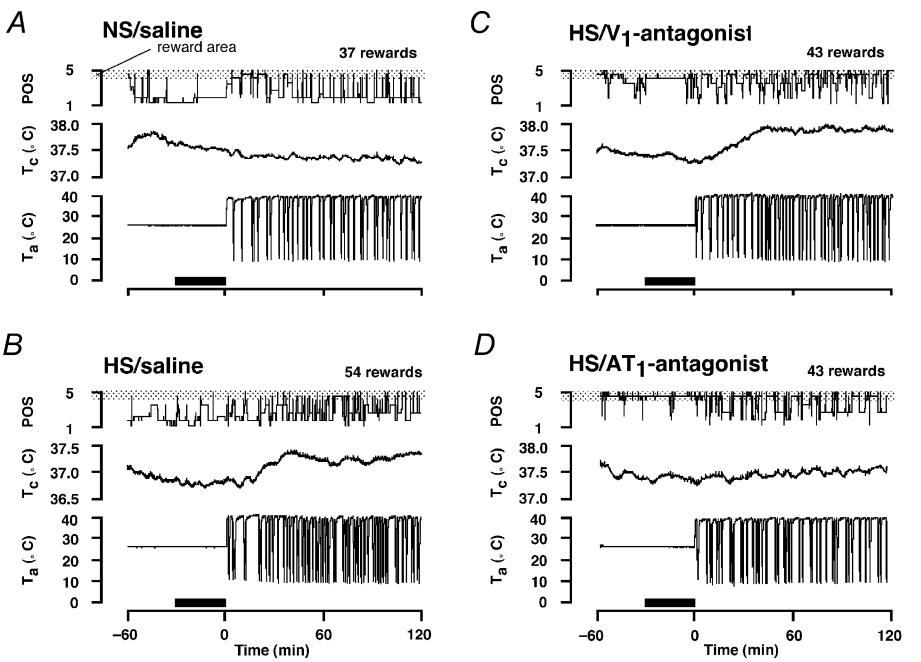

Figure 3. Examples of operant behaviour

Examples of operant behaviour in the I.C.V. saline trial in the S.C. normal-saline-injection group (154 mM, NS; *A*); the I.C.V. saline (*B*); V_1- (*C*) and AT_1-antagonist (*D*) trials in the S.C. hypertonic saline group (2500 mM, HS). The position of the rat in the behaviour box (POS), core temperature (T_{core}) and T_a were measured. A 0 °C air reward was given for 30 s when a rat entered the reward area (POS 4 or 5, dotted area in POS graph) in the behaviour box. Baseline values were determined in the latter half of the 26 °C period (horizontal bar), when measurements had stabilized.

each rat was verified histologically on brain sections after an injection of Pontamine Sky Blue (Tokyo Kasei Kogyo, Tokyo, Japan) through the cannula.

Statistical analysis

The effects of S.C. (NS and HS) and I.C.V. injections (saline and AT_1 and V_1 antagonists) and time (only when analysing the effects of heat exposure) on T_{core}, counts of operant behaviour, blood parameters and percentage change in body weight were assessed by ANOVA with repeated measurements. A *post-hoc* test to identify a significant difference at a specific time point was performed using the Newman-Keuls procedure. All values are presented as means ± S.E.M., and a null hypothesis was rejected at the level of $P < 0.05$.

RESULTS

Examples of operant behaviour in heat

Figure 3 shows examples of operant behaviour in the I.C.V. saline trial in the NS group (*A*), and saline (*B*), V_1 antagonist (*C*) and AT_1 antagonist (*D*) trials in the HS group. The rats basically stayed still at a T_a of 26 °C, and periodically moved in and out of areas four or five (reward area) in the experimental box during the 40 °C heat exposure. T_{core} became stable in the last half of the 2 h heat-exposure period. T_a dropped to about 10 °C after each 30 s of 0 °C air reward and soon returned to 40 °C.

In the preliminary experiment, we observed that under 40 °C heat (without any cold rewards), T_{core} in the rats with ligated salivary ducts surpassed 40 °C within 60 min. Therefore, operant behaviour for cold rewards was assumed to be the major process by which T_{core} is reduced at 40 °C for the rats.

T_{core} and operant behaviour in the NS group

Figure 4 illustrates the averaged T_{core} and the counts of operant behaviour in the baseline period at 26 °C and each 30 min of 2 h heat in the NS group. The baseline T_{core} and movement at 26 °C did not differ among the I.C.V. saline and AT_1 and V_1 antagonist trials. There were no differences in T_{core} and the counts of operant behaviour between the saline and V_1 antagonist trials, and T_{core} in both trials remained at the baseline level throughout the heat exposure. In the AT_1-antagonist trial, the baseline T_{core} and counts of operant behaviour were similar to those in the saline trial. However, T_{core} in the AT_1-antagonist trial tended to be lower than that in the saline trial within the last two 30 min periods of heat, although no significant difference was observed.

T_{core} and operant behaviour in the HS group

In the saline trial in the HS group, the baseline T_{core} significantly decreased by 0.9 ± 0.1°C and counts of operant behaviour increased in the heat (Fig. 5) from the levels in the same trial in the NS group (Fig. 4). However, the baseline movement and T_{core} in the heat were similar. The I.C.V. AT_1 and V_1 antagonists blocked the decrease in baseline T_{core} and the increase in counts of operant behaviour in the HS group. In the AT_1-antagonist and V_1-antagonist trials in the HS group, the baseline movement at 26 °C was not different from that in the saline trial in the NS group. In the HS group, T_{core} in the V_1-antagonist trial in the heat was higher than that in the saline trial, reaching 38.2 ± 0.2 °C, although the T_{core} in the AT_1-antagonist trial was not different from that in the saline trial.

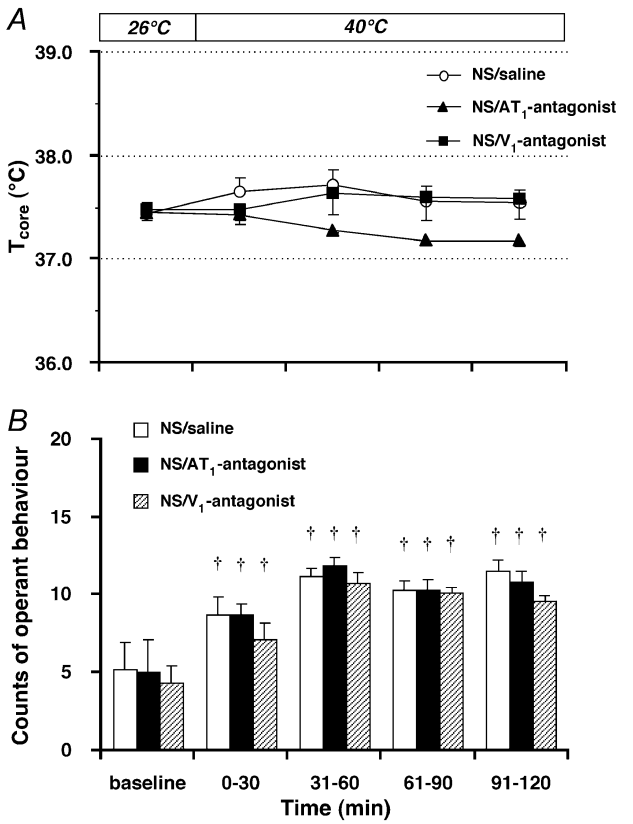

Figure 4. Changes in T_{core} (*A*) and counts of operant behaviour (*B*) for each 30 min period in the NS group

Values are means ± S.E.M. ($n = 7$ in each trial). The baseline values were taken in the last 30 min of 1 h at 26 °C. T_{core} was averaged and operant behaviour was counted during each 30 min period. †Significantly different from the baseline value in each trial, $P < 0.05$.

Table 1. Changes in haematocrit, plasma protein concentration, plasma osmolality and percentage reduction of body weight

I.C.V. injectate	Hct (%)			PPC (g dl^{-1})			$osmol_{pl}$ (mosmol kg^{-1})			body weight (Δ%)	
	-150 min	-90 min	120 min	-150 min	-90 min	120 min	-150 min	-90 min	120 min	-90 min	120 min
NS/saline	42 ± 1	37 ± 1†	39 ± 1†	6.2 ± 0.1	5.7 ± 0.1†	5.9 ± 0.1†	296 ± 1	296 ± 1	297 ± 1	-0.9 ± 0.3	-3.0 ± 0.4†
NS/AT_1-antagonist	43 ± 1	39 ± 1†	37 ± 1†	6.1 ± 0.1	5.6 ± 0.1†	5.8 ± 0.2†	300 ± 3	297 ± 2	297 ± 2	-0.3 ± 0.1	-1.5 ± 0.3†
NS/V_1-antagonist	41 ± 1	37 ± 2†	37 ± 1†	6.2 ± 0.1	5.8 ± 0.1†	5.9 ± 0.1†	298 ± 2	293 ± 3	297 ± 2	-1.3 ± 0.2	-3.0 ± 0.3†
HS/saline	42 ± 1	39 ± 1†	42 ± 1*	6.0 ± 0.1	5.4 ± 0.2†	6.2 ± 0.1*	298 ± 1	318 ± 2†*	328 ± 2†*	-1.7 ± 0.4*	-6.6 ± 0.5†*
HS/AT_1-antagonist	44 ± 1	39 ± 1†	43 ± 1*	6.3 ± 0.1	5.6 ± 0.1†	6.5 ± 0.1*	297 ± 2	312 ± 3†*	322 ± 2†*	-1.5 ± 0.2*	-5.5 ± 0.2†*
HS/V_1-antagonist	44 ± 1	39 ± 1†	43 ± 1*	6.1 ± 0.1	5.5 ± 0.2†	6.3 ± 0.2*	298 ± 1	316 ± 3†*	326 ± 1†*	-1.6 ± 0.2*	-6.0 ± 0.3†*

The S.C. injection of normal saline (NS) or hypertonic saline (HS) was conducted after the I.C.V. injection of either normal saline, an angiotensin II AT_1 receptor antagonist (AT_1-antagonist) or an arginine vasopressin V_1 antagonist (V_1 antagonist). -150 min, before the intracerebroventricular (I.C.V.) injection (baseline value); -90 min, 30 min after the S.C. saline injection; 120 min, at the end of the experiment. * Significantly different from the levels in the NS/saline, $P < 0.05$. †Significantly different from the baseline value (-150 min) in each trial, $P < 0.05$. Hct, haematocrit; PPC, plasma protein concentration; $osmol_{pl}$, plasma osmolality; Δ% body weight, percentage reduction of body weight.

Changes in Hct, PPC, $osmol_{pl}$ and percentage change in body weight

The values of Hct, PPC and $osmol_{pl}$ just before the I.C.V. injection (−150 min) were similar among all the trials in both groups (Table 1). At 30 min after the S.C. saline injection (−90 min), Hct and PPC decreased in all trials in both the NS and HS groups, and those in the HS group were restored to baseline levels by the end of experiment (120 min). The plasma osmolality increased ($P < 0.05$) only after the S.C. HS injection, but no difference among the three I.C.V. injections was observed. The percentage reduction in body weight after the S.C. saline injection was greater ($P < 0.05$) in the HS group than in the NS group. Furthermore, no difference among the three I.C.V. injections was identified in percentage change in body weight.

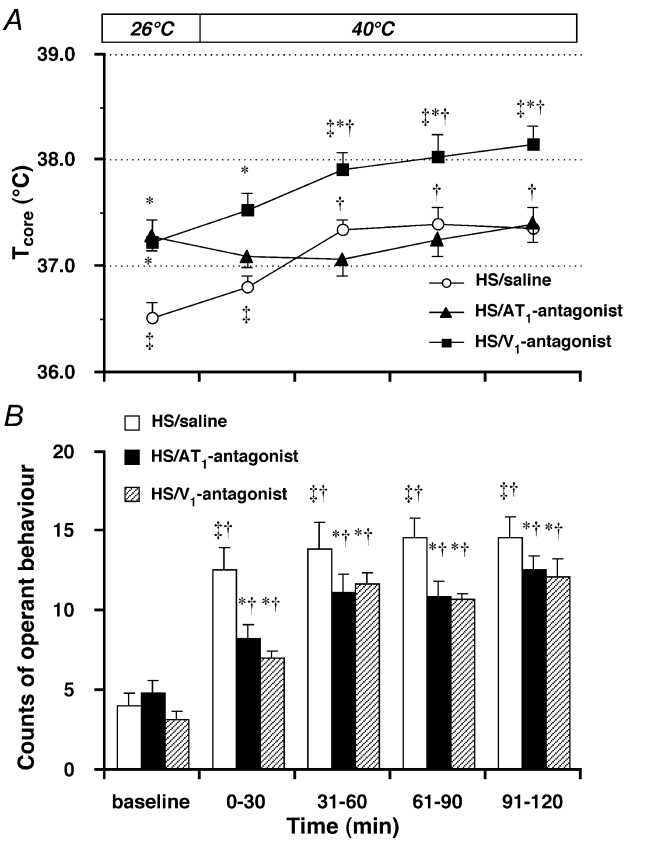

Figure 5. Changes in T_{core} (*A*) and counts of operant behaviour (*B*) for each 30 min period in the HS group

Values are means ± S.E.M. ($n = 7$ in each trial). The baseline values were taken in the last 30 min of the 1 h at 26 °C period. T_{core} was averaged, and operant behaviour was counted during each 30 min period. ‡ Significantly different from the value in the I.C.V. saline trial in the S.C. NS group, $P < 0.05$. * Significantly different from the value in the I.C.V. saline trial in the S.C. HS group, $P < 0.05$. † Significantly different from the baseline value in each trial, $P < 0.05$.

DISCUSSION

In the present study, the S.C. hypertonic saline injection reduced T_{core} at 26 °C and increased the heat-escape/cold-seeking behaviour as observed in our previous study (Nagashima *et al.* 2001). These responses were suppressed by the I.C.V. injections of both AT_1 and V_1 antagonists.

It has been reported that an increase in $osmol_{pl}$ and a decrease in blood volume are probable mechanisms affecting both autonomic and behavioural thermoregulatory responses to heat (Baker & Doris, 1982; Horowitz & Nadel, 1984; Baker & Dawson, 1985; Horowitz & Meiri, 1985; Thornton & Proppe, 1988; Nagashima *et al.* 2001). In this study, $osmol_{pl}$ increased without any differences among the three I.C.V. trials in the HS group (Table 1). The increase in blood volume after the S.C. HS, estimated from the changes in Hct and PPC (Miki *et al.* 1987), was similar among the trials (6 ± 1, 5 ± 1 and 5 ± 1 % in the I.C.V. saline and AT_1- and V_1-antagonist trials, respectively). However, the blood volume after the S.C. NS remained unchanged (0 ± 2 %, 0 ± 1 % and 1 ± 1 % in the I.C.V. saline and AT_1- and V_1-antagonist trials, respectively). Thus, neither the AT_1 nor V_1 antagonist changed T_{core} or the counts of operant behaviour by affecting $osmol_{pl}$ or blood volume.

It is supposed that the S.C. HS injection in this study increased AII and AVP in the cerebrospinal fluid (CSF). In dogs, a 5 % saline infusion increased osmolality in the plasma and CSF by 16 and 9 mosmol kg^{-1}, respectively, with an elevation of AII in the CSF by 6.4 pg ml^{-1} (Simon-Oppermann *et al.* 1986). Continuous drinking of 2 % saline in rats induced increases in osmolality and AVP in the CSF by 3 mosmol kg^{-1} and 5.5 pg ml^{-1} (Jolkkonen *et al.* 1988). These results show that the increase in plasma osmolality by ~30 mosmol kg^{-1} after the S.C. HS injection (Table 1) was sufficiently high to increase AII and AVP in the CSF.

Effects of I.C.V. AT_1 and V_1 antagonists on baseline T_{core} at 26 °C

T_{core} at 26 °C was significantly decreased in the saline trial in the HS group (Fig. 5*A*). This response was suppressed by the I.C.V. AT_1 and V_1 antagonists. Since neither antagonist altered the baseline T_{core} in the NS group, the antagonists themselves are not likely to have increased the T_{core}. It has been reported that I.C.V. injections of AII (Shido & Nagasaka, 1985; Rohmeiss *et al.* 1995) and AVP (Naylor *et al.* 1986) induce hypothermia in rats. Thus, the reduction in T_{core} at 26 °C in the HS group could be associated with the increase in AII and AVP in the brain.

Effects of I.C.V. AT_1 and V_1 antagonists on heat-escape/cold-seeking behaviour

Systemic salt loading is a strong stimulus increasing operant heat-escape/cold-seeking behaviour in rats (Nagashima *et al.* 2001). Indeed, the counts of operant behaviour after I.C.V. saline were greater in the HS group than in the NS group (Fig. 5*B*). Since T_{core} after the I.C.V. saline was kept lower in the HS group than in the NS group (Fig. 5*A*), the increase in operant behaviour was not a secondary response to an increase in T_{core} due to reduced autonomic heat dissipation, but a direct response to salt loading. In the present operant system, rats could obtain cold air by non-specific movements to the reward area. However, we have shown previously that S.C. HS injection did not increase activity in general, and rats moved less when cold air was automatically given regardless of their movement (Nagashima *et al.* 2001). Although the baseline movement at 26 °C was similar in both I.C.V. saline trials in the NS and HS groups, the movement at 40 °C was greater in the HS group than in the NS group. Therefore, the observed increase in movement in the operant system for these rats might be determined, at least partly, by thermoregulatory demand.

The I.C.V. injections of AT_1 and V_1 antagonists suppressed the increase in operant behaviour induced by salt loading (Fig. 5*B*). In the HS group, T_{core} in both the AT_1- and V_1-antagonist trials during the heat exposure was never lower than in the saline trial (Fig. 5*A*). Therefore, the effects of the antagonists are not a secondary response to a reduction in T_{core}. Moreover, these antagonists had no effect on the movement in the NS group (Fig. 4*B*), which indicates that they have no direct influence on operant behaviour. Therefore, the increase in operant behaviour is mediated by endogenous AII and AVP in the brain via the AT_1 and V_1 receptors, respectively.

Roberts (1988) suggested that behavioural responses to heat, such as grooming or body extension, were influenced by thermal inputs from the body core and skin. It is not clear how these inputs determine operant behaviour in the present study. However, T_{core} was similar in the saline trials in the NS and HS groups during the heat exposure. This result may suggest that salt loading strengthens the sensitivity to the thermal input from the skin, thus activating operant behaviour. A study using a thermocline may help to verify this speculation.

Relationship between AII and AVP

It is well known that centrally acting AII is closely associated with AVP secretion as well as natriuretic, dipsogenic and pressor responses following salt loading (Zucker & Kalay, 1976; Thrasher *et al.* 1980; Hogarty *et al.* 1994; McKinley *et al.* 1994; Rohmeiss *et al.* 1995). In addition, these responses are blocked by I.C.V. AT_1 antagonists (Kregel *et al.* 1994; McKinley *et al.* 1994; Rohmeiss *et al.* 1995; Mathai *et al.* 2000). It was reported that AVP levels in the CSF were well correlated with that in the plasma (Szczepanska-Sadowska *et al.* 1983). Therefore, in the study presented here, the I.C.V. AT_1 antagonist

probably suppressed the increases in both central and plasma AVP in response to salt loading. Indeed, the single application of AT_1 or V_1 antagonist abolished both the decrease in T_{core} at 26 °C and the increase in operant behaviour observed after salt loading. Thus, the effects of the I.C.V. AT_1 antagonist were probably produced by the suppression of AVP secretion.

Effects of I.C.V. AT_1 antagonist and V_1 antagonist on T_{core} during 40 °C heat exposure

The effects of the I.C.V. AT_1 and V_1 antagonists on T_{core} in the heat seemed to be different, although both antagonists similarly suppressed the decrease in T_{core} at 26 °C and the increase in operant behaviour. It is arguable, therefore, that in the HS group during the heat exposure, T_{core} in the V_1-antagonist trial remained higher than in the saline trial as a result of the smaller counts of cold-air rewards (Fig. 5*A*, *B*). In contrast, T_{core} in the AT_1-antagonist trial did not increase, even with the smaller counts of cold-air rewards. It is notable that the I.C.V. AT_1 antagonist also tended to influence T_{core} in the NS group, although the effect did not reach a significant level (Fig. 4*A*). These results may indicate that the I.C.V. AT_1 antagonist was able to effect a reduction in T_{core} in the latter half of the 2 h heat exposure by modifying some autonomic thermoregulatory mechanisms, although these mechanisms remain to be elucidated. Moreover, AVP need not be involved in the response. However, this effect might be counterbalanced by an increase in heat gain due to the reduction in counts of cold-air rewards in the HS group, maintaining T_{core} at baseline levels during heat exposure. These results suggest that the brain AII induced by salt loading is an important modulator in controlling various autonomic and behavioural processes for thermoregulation in response to osmotic stimulation. However, the exact roles and precise mechanisms need to be elucidated in future studies.

In conclusion, the I.C.V. injections of both AT_1 and V_1 antagonists suppressed the reduction in T_{core} at 26 °C and the increase in operant heat-escape/cold-seeking behaviour in response to systemic salt loading. These results suggest strongly that AII and AVP in the brain are key substances in modulating autonomic and behavioural thermoregulatory processes during osmotic stress.

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