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Cold-Induced Salt Intake in Mice and Catecholamine, Renin and Thermogenesis Mechanisms

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Cold induces increased intake of salt in mice. To examine involvement of renin and catecholamines, male ICR mice were exposed to cold (7–9°C; 6 h/day; 4 days), and half of them were allowed to choose between water and 0·9% NaCl. Plasma renin activity (PRA) and catecholamine concentrations in plasma, adrenal gland, kidney, brown adipose tissue (BAT) and brain were examined in three phases: for 9 h before exposure to cold, during 6 h of cold exposure and for 9 h after the exposure. The amount of salt intake from NaCl solution and from food, PRA and noradrenaline (NE) concentrations in kidney and medulla oblongata were higher during cold and the 9 h after exposure to cold than during the 9 h before the exposure. These results are consistent with the suggestion that cold-induced catecholamine metabolism enhanced activity in the renin–angiotensin system, which played an important role in the arousal of salt appetite. During cold exposure, concentrations of NE and dopamine in BAT were higher in mice with access to NaCl solution than those without NaCl to drink. These results suggest that cold-induced salt intake enhanced non-shivering thermogenesis, and are consistent with our previous report that high salt intake helped to maintain colonic temperature under cold exposure.

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INTRODUCTION

Repeated short-term exposure to cold (6 h/day for 4 days) induced an increase in daily salt intake in mice allowed to choose between water and 0·9% NaCl to drink (Dejima *et al.*, 1991). The source of cold-induced salt intake was increased intake of 0·9% NaCl solution and of food, not only during the 6 h of cold but also during 9 h of normal temperature after cold exposure. However, the physiological mechanisms of this phenomenon have been unclear.

Total fluid intake (NaCl solution and distilled water) also increased during 9 h after exposure to cold in groups of mice both with and without access to NaCl solution (Dejima *et al.*, 1991). This result was consistent with the increased levels of renin activity and angiotensin II concentration in plasma observed during thermogenic-drinking in rats (Fregly & Waters, 1966; Katovich *et al.*, 1979; Nelson *et al.*, 1975). A direct role of the renin–angiotensin system (RAS) in the arousal of salt intake has been established (Avrith & Fitzsimons, 1980; Fregly *et al.*, 1985; Rowland & Fregly, 1988; Sakai *et al.*, 1986). It is also known that noradrenaline stimulates renin biosynthesis (Bunag *et al.*, 1966; Johnson *et al.*, 1971; Vander, 1965) and that cold exposure activates catecholamine metabolism as a thermogenic response (Janský *et al.*, 1967; Janský, 1969; Leduc, 1961; Murazumi, 1988). These findings suggest that the RAS stimulation by cold-induced catecholamine activation was related to the increase of NaCl solution intake in mice during 9 h after cold exposure in our previous experiment (Dejima *et al.*, 1991).

In that report, it was also observed that colonic temperature in the cold was significantly lower in mice given only distilled water than those given NaCl solution. High-salt-intake-induced sodium pump activity involves NST and vasoconstriction (Blaustein, 1977; Bradlaugh *et al.*, 1984, 1987; DeWardener *et al.*, 1981; Guernsey & Stevens, 1977; Guernsey & Whittow, 1981; Stevens & Kido, 1974; Swann, 1985). High salt intake increases post-absorptive resting metabolic rate in humans (Otaga, 1948; Ogata *et al.*, 1952) and tissue respiration in rats and rabbits kept in a cold room (Maeda, 1957a, 1957b, 1957c).

Thus, we hypothesize that the physiological mechanisms by which cold temperature induces salt appetite are as follows: (1) cold-induced catecholamine metabolism enhances RAS activity; (2) activated RAS plays an important role in the arousal of salt appetite during normal temperature after cold exposure; (3) high salt intake enhances NST and/or vasoconstriction during cold exposure on the next day and that prevents mice from decreasing their colonic temperature. To test this hypothesis, the present study was designed as follows.

Renin is secreted from the kidneys into blood (Semple *et al.*, 1979; Tigerstedt & Bergman, 1898) under the control of catecholamines in the blood (Ball *et al.*, 1981; Johnson *et al.*, 1979; Ueda *et al.*, 1970), released from renal sympathetic nerve endings (Johnson *et al.*, 1971). Therefore, catecholamine concentrations in the plasma and kidneys, together with enhanced RAS, were measured.

Activation of RAS can be estimated by elevation of plasma renin activity (PRA) (Bergen *et al.*, 1992; Semple *et al.*, 1979; Tree *et al.*, 1984). The hypothesis would be supported if NaCl solution intake were increased during normal temperature after cold exposure and PRA were higher in that period than in the cold-exposure period.

Development of NST is mediated by temperature-sensitive neurons in hypothalamus (Banet & Hensel, 1976), and also in midbrain and medulla oblongata but not in cerebral cortex and cerebellum (Hori & Harada, 1976; Ingenito, 1968;

Inoue & Murakami, 1976; Lipton, 1973; Nakayama & Hardy, 1969). NST appears in brown adipose tissues (BAT) of mice and rats (Dubois-Ferrière & Chinet, 1981; Foster & Frydman, 1979). Noradrenaline is the activator of NST in BAT (Kuroshima *et al*., 1984; Schönbaum *et al*., 1966). Therefore, catecholamine levels in hypothalamus, midbrain, medulla oblongata and BAT were compared between mice with and without NaCl to drink.

METHOD

Animals and Apparatus

Male ICR mice (Charles River Japan Inc., Hino), 10 weeks of age (mean body weight: 36 g), were used for all the experiments. Mice were housed individually in polycarbonate cages with stainless steel net floors, to avoid the effects of huddling on colonic temperature during the cold, with 12 h of light (0800–2000 hrs) and 12 h of dark (2000–0800 hrs), at a room temperature between 20°C and 22°C. Two water-supplying tubes were set on each cage, and the mouse was allowed to drink water or NaCl solution from either of them. The design of the apparatus and the method of controlling interapparatus and interposition variation were described in our previous paper (Dejima *et al*., 1991). Mice were given *ad libitum* access to food (Type MF; Oriental Farm Co., Tokyo) containing 2·2 mg/g of sodium and 8·7 mg/g of potassium, according to the measurements by the authors.

Experimental Design

The experiment was performed over eight consecutive days (Fig. 1): period 1 was 4 days in which the mice were kept at normal temperature (20–22°C), and period 2 was 4 days in which they were exposed to cold for 6 h/day.

Forty-eight mice were used for each experiment. As all experimental procedures mentioned below were conducted twice, 96 mice were used in total. For half of the mice, both drinking tubes were filled with distilled water (group W); for the other half, one tube was filled with distilled water and the other with 0·9% NaCl solution (group Na).

Body weight was measured every day at 1000 hrs. At 1700 hrs, food pellets and fluids were changed.

Intakes of food and fluid (water and NaCl solution, separately) were measured every day at the end of each of the three observation times, i.e. 0200–1100 hrs, 1100–1700 hrs, and 1700–0200 hrs. The amounts of 24-h food and fluid intakes were calculated from 1100 hrs. In group Na, total fluid intake and preference for 0·9% NaCl solution were calculated as follows (Kare, 1961; Kare & Henkin, 1969; Pfaffmann, 1952): (Total fluid intake) = (Distilled water intake) + (0·9% NaCl solution intake); (Preference for 0·9% NaCl solution) = (0·9% NaCl solution intake)/(Total fluid intake) × 100.

Sixteen mice, eight belonging to group W and eight to group Na, were decapitated at 1630–1700 hrs on the last day of period 1 (phase NT). During period 2, the remaining 32 mice were transferred every day at 1100 hrs from a room at normal temperature (20–22°C) to an air-conditioned room (Meluko, Tokyo) of a mean temperature of 8°C (7–9°C) and humidity of 50%; after 6 h of cold exposure

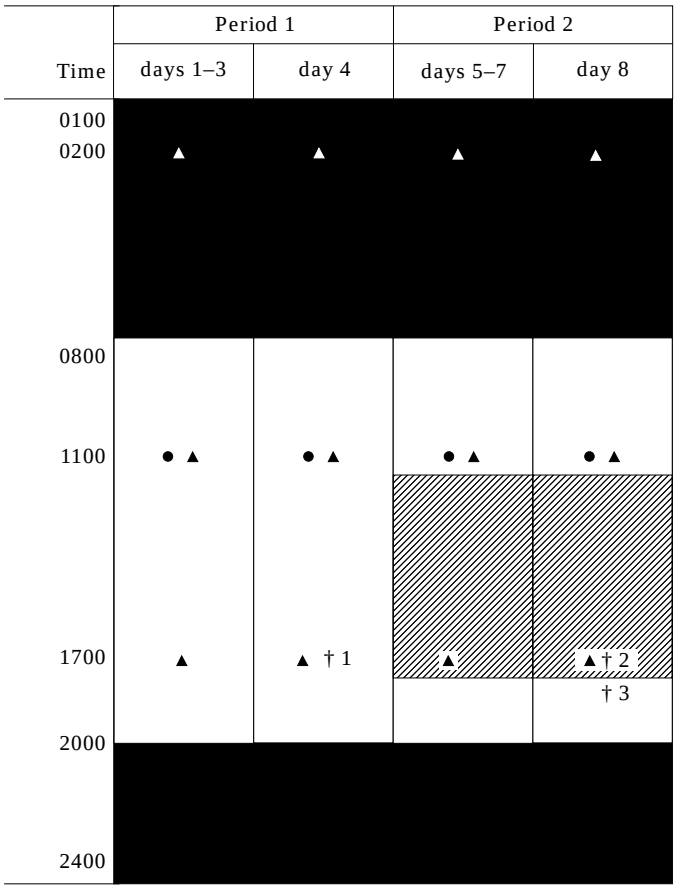

FIGURE 1. Experimental design. ■, dark; ▨, cold; □, light; ●, measurement of body weight; ▲, measurement of food and fluid intake; †1, mice killed (phase NT); †2, mice killed (phase C); †3, mice killed (phase PC).

(1100–1700 hrs), the mice were returned to the room at normal temperature. Loss of water or saline through dripping due to increase of internal pressure of the tube when the mice were transferred from the cold room to the room of normal temperature was controlled, and no specific effects of moving, handling and environmental difference between the two rooms except temperature were expected as mentioned in our previous report (Dejima *et al*., 1991). Of these, 16 mice, eight belonging to group W and eight to group Na, were killed at 1630–1700 hrs (during cold exposure) on the last day of period 2 (phase C), and the other 16 mice, eight belonging to group W and eight to group Na, were killed 15–30 min after cold exposure (phase PC).

Blood was taken at the time of death in order to determine plasma catecholamine concentration and plasma renin activity (PRA). However, in one of the experiments, the brain was removed and, in another, adrenal glands, 0·3–0·5 g of BAT, and kidneys were taken; in samples from these organs, catecholamine (noradraneline, NE; adrenaline, E; and dopamine, DA) concentrations were analysed. In this experiment, colonic temperature was not measured to avoid the effects of insertion

of anal thermistor probe on catecholamine levels; the relationship between colonic temperature and NaCl intake had been established in our previous study.

Catecholamine Analysis

Reagents and standards. The extract solution (ES), which consisted of 0·4 N perchloric acid, 7·9 mM $Na_2S_2O_5$, and 1·3 mM EDTA·2Na, was used for extraction of catecholamine from tissues and plasma (Wagner *et al.*, 1982). This ES was also used as the standard stock solution. For the standard, NE, E, DA and DEP (Sigma, U.S.A.) were dissolved into ES. Tris–HCl used was 1 M tris-(hydroxymethyl)-aminomethane solution adjusted to pH 8·6 by hydrochloric acid.

Sample preparation for plasma. The mice were decapitated and the first 1 ml of drained blood was collected into a tube with 1 mg of EDTA·2Na. The blood was cooled by immersion in an ice bath just after collection and centrifuged at 4000 **g** for 15 min at 4°C, and the plasma portion was taken. Then, 0·4 ml ES solution containing 250 ng DEP as internal standard was added to 0·4 ml plasma and, after centrifugation at 15 000 **g** for 10 min at 4°C, the supernatant was withdrawn. The pH of the supernatant was adjusted at 8·3 with Tris–HCl and shaken for 25 min with 50 mg of alumina (02084, Akt I from ICN, Germany) which was purified and activated by the methods of Anton and Sayre (1962) before use. After washing the alumina four times with distilled water, 200 µl of ES was added and vigorously shaken for 1 min in ice cold. The extraction was filtered through a 0·45-µm pore size membrane filter (PVDF, Millipore Ltd., Japan) and then injected to HPLC.

Sample preparation for organs and brain section. The brain was frozen in liquid nitrogen immediately after decapitation. The brain was dissected into five regions, i.e. cerebellum, medulla oblongata, hypothalamus, midbrain, and cerebral cortex, according to the method of Glowinski and Iversen (1966). Adrenal glands, BAT, and kidneys were taken and immediately frozen in liquid nitrogen. The amounts of internal standards (DEP) added were, respectively, 2000 ng to whole adrenal glands, 250 ng to 0·3–0·4 g of BAT, 250 ng to whole kidney, 15 ng to either cerebellum, medulla oblongata, or midbrain, 5 ng to hypothalamus, and 150 ng to cerebral cortex. Then, each organ or brain region was homogenized with ES by supersonic homogenizer (Taitec Co., Japan) in ice cold. After centrifugation at 15 000 **g** for 10 min, the supernatant was filtered through a 0·45 µm membrane filter and then injected to HPLC.

Mobile phase. Mobile phase for HPLC consisted of 0·1 M NaH_2PO_4, 0·02 M trichloroacetic acid, 0·7 mM SDS (sodium dodecyl sulfate; electrophoretic grade from Bio-Rad Laboratories, U.S.A.), 0·03 mM EDTA·2Na, 10% acetonitrile and 5% methanol. This solution was prepared by dissolving 15·6 g of NaH_2PO_4 and 3·3 g of trichloroacetic acid in 1000 ml of Milli-Q-Plus (Millipore) purified water. The pH of this solution was adjusted at 3·10 with NaOH. This mixture was filtered through a 0·22 µm GV membrane filter (Millipore) with vacuum pump. Then, 202 mg of SDS and 11 mg of EDTA·2Na were added. Finally, 117·6 ml of acetonitrile and 58·8 ml of methanol were added and thoroughly mixed. The mobile phase was deaerated with helium and pumped at a rate of 1·5 ml/min.

HPLC System. The HPLC system consisted of a model LC-10AD liquid pump (Shimadzu Co., Japan) equipped with a 0·45 μm inlet filter. Samples were introduced through a model SIL-10A auto-injector with a sample cooler (Shimadzu), which was set at 4°C and at 50 μl of sample holding loop. This injection system was controlled by a model SCL-10A (Shimadzu). Separations were performed on the C_{18} reverse-phase column (150 mm × 4·6 ϕ mm; MC Medical Inc., Japan) with the C_{18} pre-column (μBondapak; Waters Co., Japan). Temperatures of these columns were maintained at 45°C in a model CTO-10A column oven (Shimadzu). The detection system used was a model 5200A (ESA, U.S.A.) electrochemical detector (Kissinger, 1977; Kurata *et al.*, 1987) which consisted of a coulometric conditioning cell (model 5021) and a high-sensitivity analytical cell (model 5011). The working electrode potentials for these cells were −50 mV and +300 mV, respectively (Takeda *et al.*, 1990).

Plasma Renin Activity (PRA) Analysis

The mice were decapitated and the first 1 ml of drained blood was collected into a tube with 1 mg of EDTA·2Na. The blood was cooled by immersion in an ice bath immediately after collection and centrifuged at 1200 **g** for 10 min at 4°C, and the plasma was taken. The plasma was stored at −80°C until analysis (within 48 h). Plasma renin activity (PRA) was determined using an angiotensin I (^{125}I) radio-immunoassay kit (Renin·Riabead; Dainabot Co., Japan) (Haber *et al.*, 1969; Ikeda *et al.*, 1981). For calculation of the data, the log–logit linearization was applied (Rodbard *et al.*, 1969; Rodbard, 1974).

Statistical Analysis

Normality of distribution of data was examined by Kolmogorov–Smirnov test. For preference for 0·9% NaCl solution, arc-sine conversion was applied to fit the normal distribution. One-way analysis of variance was used to test the effects of experimental days in each period. Since there were no significant changes in body weight and food and fluid intakes throughout the 4 days in each period in both experiments, the data for each period were pooled for analyses.

Two-way analysis of variance was applied to the effects of time periods and drinking fluids. When the result of the ANOVA test was significant, Tukey's multiple-range test was further applied to compare the differences in mean values. Student's *t*-test was used to examine the effects of cold exposure on amounts of intake of food, fluid and sodium, and on preference for 0·9% NaCl solution (in period 2).

For catecholamine levels and renin activity, two-way ANOVA was applied to test the effects of the three phases of death (NT, C and PC) and of drinking fluids. When the ANOVA gave significant differences, Tukey's multiple-range test was applied to compare the mean values. Student's *t*-test was also conducted to compare the measurement values at each phase.

RESULTS

Body weight, food intake, fluid intake, preference for 0·9% NaCl solution, and sodium intake in each group of mice were the same as those observed in our previous study (Dejima *et al.*, 1991).

Body weight did not differ significantly among the groups in this study nor did it change significantly in any group throughout the experimental period.

Food, Fluid and Sodium Intakes

Intakes over 24 h. In both periods 1 and 2, sodium intake was higher in group Na than in group W (Table 1). Food intake, sodium intake, intake of NaCl solution and preference for NaCl solution were higher in period 2 than in period 1.

Intakes and preference at each observation time. Food intake was higher in period 2 in both group W and group Na at the observation time of 1100–1700 hrs, and higher in period 2 in only group Na at 1700–200 hrs (Fig. 2A). Food intake at 1100–1700 hrs was significantly higher in group W than in group Na in period 2. Total fluid intake at the observation time of 1700–200 hrs was higher in period 2 in both group W and group Na (Fig. 2B). Also, the amount of 0·9% NaCl solution intake was higher in period 2 than in period 1, only at the observation time of 1700–200 hrs. During all the observation periods, the preferences in period 2 were significantly higher than those in period 1 (Table 2). Increased sodium intake in period 2 was observed at 1100–1700 hrs in both groups W and Na, and at 1700–200 hrs in group Na only (Fig. 2C). The sodium intake at 1100–1700 hrs did not differ between group W and group Na; this was because a large amount of sodium came from food.

Plasma Renin Activity (PRA) and Catecholamine Concentrations in Kidney and Medulla Oblongata

PRA changed significantly throughout the three phases in both group W and group Na (Fig. 3A). In phase NT, PRA did not significantly differ between the groups but, in phase C, PRA was significantly higher than that at phase NT for group Na but at the same level as phase NT for group W. In phase PC, PRA in group Na remained at high levels, whereas that in group W significantly increased from phase C. This changing pattern was similar to that of NE concentration in kidney (Fig. 3B) and medulla oblongata (Fig. 3C). Adrenaline and DA levels in these organs were not associated with PRA (data are not shown).

Catecholamine Concentrations in Adrenal Gland and Plasma

Catecholamine levels in the adrenal gland (Fig. 3D, E and F) increased significantly from phase NT to phase C and remained unchanged until phase PC but did not significantly differ between the groups. In plasma, all catecholamine levels (Fig. 3G, H and I) were significantly higher in group Na than in group W at phases C and PC. All the catecholamine levels were significantly higher at phase C than at phase NT in both groups. Furthermore, at phase PC, only NE in both groups and E in group Na had significantly higher levels than at phase C.

Catecholamine Concentrations in Hypothalamus and BAT

In hypothalamus, concentrations of NE (Fig. 3J) and DA (Fig. 3L) changed similarly. The levels in group Na were almost flat, whereas those in group W were

TABLE 1
Food, fluid and sodium intake and preference for 0·9% NaCl solution over 24 h

		Group (solution)	*n*	Period	
				1	2
Food intake		W (0% & 0%)	8	4·92 ± 0·93	6·43 ± 0·72*
		Na (0% & 0·9%)	8	4·96 ± 1·01	6·29 ± 0·63*
	ANOVA	(Period): $F(1,28) = 23·111$; $p < 0·01$, (Group): $F(1,28) = 0·029$; NS			
		(Interaction): $F(1,31) = 0·109$; NS			
Total fluid intake		W (0% & 0%)	8	5·73 ± 0·64	5·99 ± 0·68
		Na (0% & 0·9%)	8	5·96 ± 1·07	5·72 ± 0·08
	ANOVA	(Period): $F(1,28) = 0·001$; NS, (Group): $F(1,28) = 0·004$; NS			
		(Interaction): $F(1,31) = 0·992$; NS			
Intake of 0·9% NaCl solution		Na (0% & 0·9%)	8	2·05 ± 0·66	3·40 ± 0·20*
		t-test $t(14) = 5·478$; $p < 0·01$			
Preference for 0·9% NaCl solution		Na (0% & 0·9%)	8	35·18 ± 11·03	59·37 ± 3·83*
		t-test $t(14) = 5·481$; $p < 0·01$			
Total sodium intake from food and NaCl solution		W (0% & 0%)	8	11·51 ± 2·09	14·85 ± 1·69*
		Na (0% & 0·9%)	8	18·53 ± 2·87†	25·85 ± 1·50*†
	ANOVA	(Period): $F(1,28) = 51·279$; $p < 0·01$, (Group): $F(1,28) = 146·613$; $p < 0·01$			
		(Interaction): $F(1,31) = 7·110$; $p < 0·05$			

Values show mean ± *SD* of food intake (g), total fluid intake (g), intake of 0·9% NaCl solution (g), preference for 0·9% NaCl solution (%), and total sodium intake from food and NaCl solution (mg) over 24 h. Values marked are significantly different from period 1 (*), and from group W (†) by Tukey's multiple range test or *t*-test.

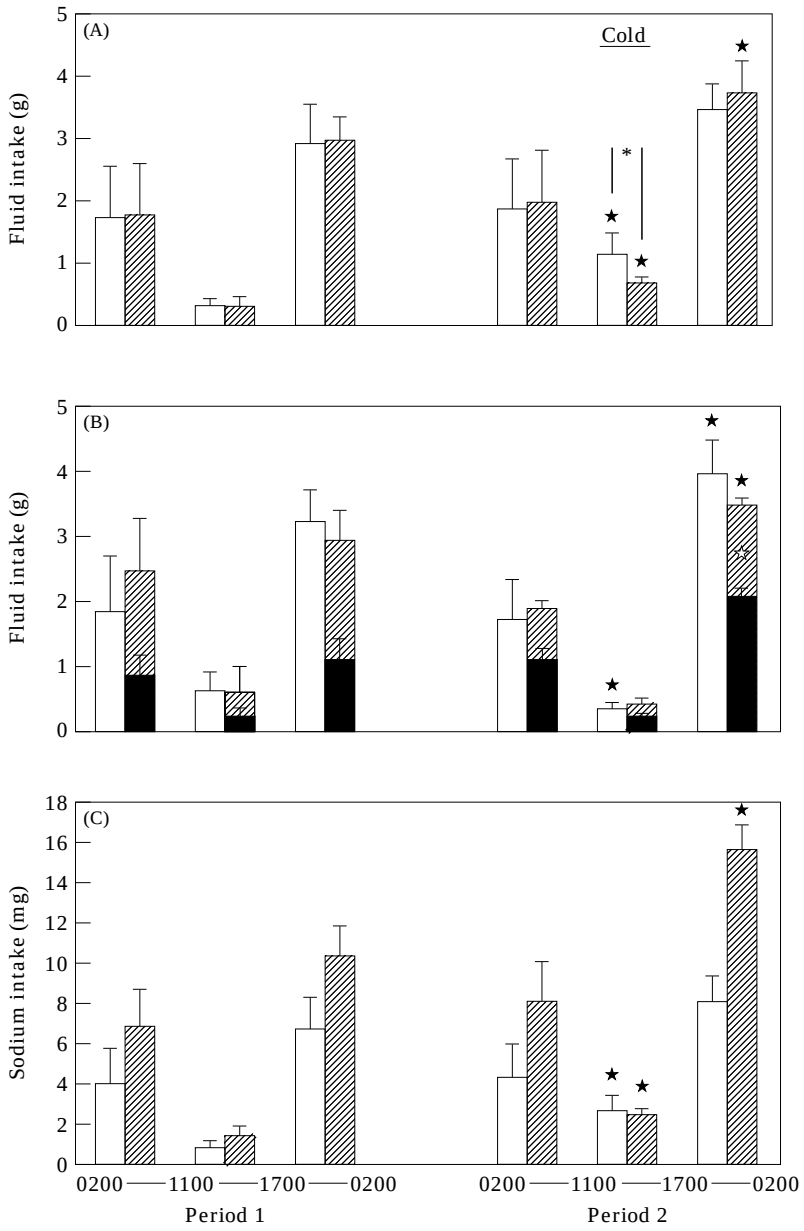

FIGURE 2. Mean and *SD* of intakes of (A) food, (B) fluid and (C) sodium, at each observation time (0200–1100, 1100–1700, 1700–0200). (A) and (C): □, group W; ▨, group Na. (B): □ and ▨, distilled water; ■, NaCl solution. , $p<0{\cdot}05$ for food, total fluid and sodium intake, and , $p<0{\cdot}05$ for NaCl solution intake in period 2 are significantly different from corresponding values in period 1 by *t*-test. *Significantly different by *t*-test ($p<0{\cdot}05$).

significantly higher in phase C than at phases NT and PC. It is also noticed that in phase C, the levels did not significantly differ between the groups. In BAT, concentrations of NE (Fig. 3M) and DA (Fig. 3O) also changed similarly. In group W,

TABLE 2
Preference for 0·9% NaCl solution at each observation time

	n	0200–1100	1100–1700	1700–0200
Period 1	8	36·82 ± 15·62	36·63 ± 14·15	36·20 ± 8·92
Period 2	8	57·45 ± 7·68*	60·87 ± 4·86*	60·33 ± 3·61*

In period 1, mice were kept in normal temperature, and in period 2, mice were exposed to cold between 1100 and 1700 hrs every day.

Values show mean ± *SD* of preference for 0·9% NaCl solution (%) in group Na. Values marked * in period 2 are significantly different ($p<0·05$) from the corresponding values in period 1. Statistical *t*-tests were made after arcsin conversion of the data.

those levels did not significantly change throughout the three phases, while those in group Na were significantly higher at phase C than at phases NT and PC. Adrenaline concentrations in hypothalamus (Fig. 3K) and in BAT (Fig. 3N) were significantly higher at phase C than at phase NT in both groups, and were higher at phase PC than at phase C. A significant intergroup difference existed only in the level for hypothalamus at phase PC.

Catecholamine Concentrations in Other Brain Regions

In cerebral cortex and cerebellum, no catecholamine (NE, E, and DA) level changed throughout the three phases in any group of mice, and there were no significant intergroup differences at any phase. In midbrain, changes of catecholamine concentration were inconsistent with the changes of salt intake (data are not shown).

DISCUSSION

Salt Intake Before Exposure to Cold

Among the normal-temperature phase before exposure to cold, preference for NaCl solution was about 30–40% in the group drinking saline but plasma renin activity did not differ between saline and water groups. This preference level was identical with the results of the authors' previous studies on the preference for NaCl solution in mice at normal temperature and under no stress (Dejima & Suzuki, 1991; Liu *et al.*, 1991). Thus, this sodium intake (18·4 mg/day) is ineffective at stimulating the renin–angiotensin system.

Both norepinephrine and dopamine levels in the hypothalamus were higher in the saline-drinking mice. Infusion of angiotensin II into the blood or cerebral ventricles decreases catecholamine levels in the hypothalamus (Fernández & Domínguez, 1991; Kawamura, 1972; Palaic & Khairallah, 1968). Since the brain renin–angiotensin system was not activated during the initial phase at normal temperature, the increased concentration of NE and DA in hypothalamus was induced directly by intake of NaCl solution; the brain sodium pump is activated by high salt intake, mediated by NE (Swann, 1983, 1984, 1985).

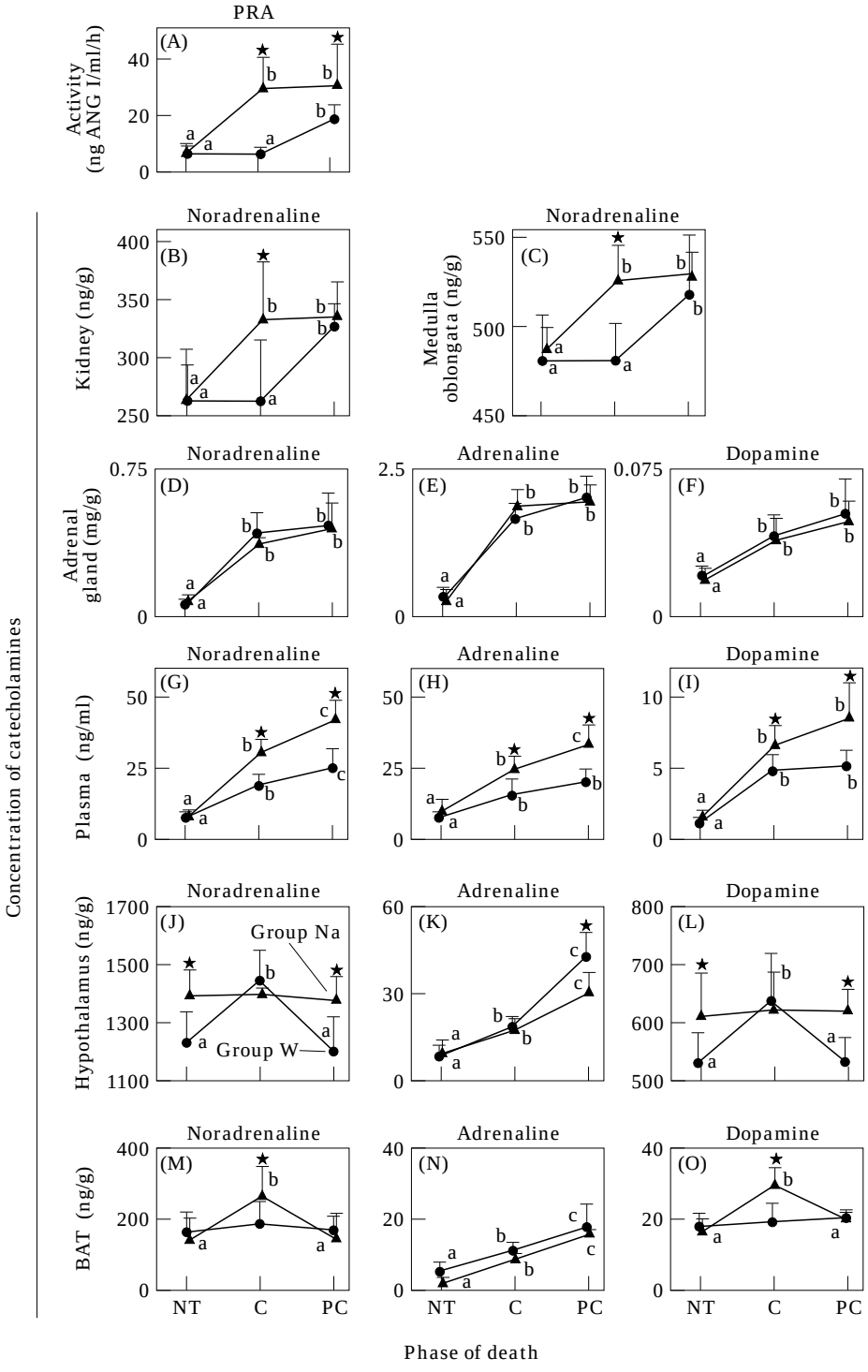

FIGURE 3. Mean and *SD* of PRA and catecholamine concentrations in various organs. ▲, group Na; ●, group W. Different letters, a, b and c, for each phase's measurements are significantly different ($p<0{\cdot}05$) by Tukey's multiple range test. Marks with are significantly different ($p<0{\cdot}05$) between the groups by *t*-test.

Enhancement of Plasma Renin Activity by Cold-Induced Catecholamine Metabolism

There are two possible mechanisms for the relationship between catecholamines and PRA enhancement.

The first mechanism, i.e. the circulating catecholamines in the blood, which may play relatively indirect roles, can be considered from the following results: concentrations of all catecholamines (NE, E, and DA) in plasma were higher at phases C and PC than at phase NT in both groups on the one hand and were higher in group Na than in group W on the other hand. It has been recognized that, among the catecholamines circulating in the blood, only E mediated by β-receptor has been confirmed to be effective on PRA enhancement (Johnson *et al.*, 1979), because DA is not effective within the physiological dose range (Ball *et al.*, 1981) and the effect of NE is still controverted; some reports indicate that NE mediated by α-receptor increases renal renin release (Bunag *et al.*, 1966; Reid *et al.*, 1978; Ueda *et al.*, 1970), but other reports indicate that NE stimulates renin release with α-adrenergic blockade (Vandongen & Peart, 1974). In the present results, not only E level but also NE and DA levels in plasma showed changing patterns, which differed from those of PRA, so that it is judged that catecholamines not only in plasma but also in the other route were the enhancer of PRA.

The next consideration is focused on the second and more direct mechanism of control of renal renin secretion, i.e. local release of catecholamines from the renal sympathetic nerve endings in juxtaglomerular cells (Barajas & Müller, 1973; Reid *et al.*, 1978; Wagermark *et al.*, 1968). This mechanism has been recognized as follows: sympathetic NE plays a direct role in renin secretion (Johnson *et al.*, 1971) mediated by β-adrenergic mechanism (Nolly *et al.*, 1974; Ueda *et al.*, 1970), which occurs even when renal vasoconstriction mediated by α-receptors is blocked (Vandongen *et al.*, 1973; Vandongen & Peart, 1974). In the present results, changing patterns of PRA and NE levels in kidney were similar throughout the experiment. Therefore, sympathetic NE mediated by β-adrenergic mechanism is judged to be a main enhancer of PRA.

Salt appetite induced by catecholamine response to cold. Salt preference and plasma renin were both enhanced during the cold at 1100–1700 hrs and during normal temperature at 0200–1100 hrs and 1700–0200 hrs. Angiotensin II penetrates the blood–brain barrier in cerebral ventricular area (Fernández & Domínguez, 1991; Kawamura, 1972; Palaic & Khairallah, 1968). Administration of norepinephrine into the hypothalamus induces salt appetite, mediated by brain renin–aiotensing (Chiaraviglio & Taleisnik, 1969; Chiaraviglio, 1976). Hence, the present results suggest that cold-induced sympathetic NE activation enhanced PRA, and then activation of RAS, continuing after exposure to cold, induced increased salt intake.

Catecholamines and Thermoregulation

The present results indicate that both thermogenic activation and high salt intake enhance non-shivering thermogenesis, and thus prevent a decrease in colonic temperature in the cold.

Cold temperature is sensed by the preoptic and anterior-hypothalamus area in the brain (Nakayama *et al.*, 1961), where catecholamine neurons are distributed (Ruggiero *et al.*, 1984). With cold exposure, NE concentration increases and its

turnover in hypothalamus is shortened (Simmonds, 1969), and development of NST is mediated by the hypothalamic temperature-sensitive neurons (Banet & Hensel, 1976). A high level of catecholamine in hypothalamus was observed in the present study. Norepinephrine and DA levels in hypothalamus remained at high levels throughout the three phases in the drinking saline group, while the mice drinking water had higher hypothalmic catecholamines in the cold than in either phase at normal temperature. These results suggest that high levels of NE and DA in hypothalamus play a significant role in enhancement of NST in the cold.

Adrenaline enhances NST in BAT, since large thermogenesis is observed after the administration of NE and E into the intrascapular region in mice (Mejsner & Janský, 1971). However, Leduc (1961) suggested that E is not needed unless the NE mechanism becomes saturated and thus is judged to play a secondary role as a defense against cold. In the present results, E level in BAT was higher in the cold than beforehand in both saline and water groups, though there was no difference between groups. These results suggest that, although E contributes to inducing NST in cold, the enhancement of NST with high-salt intake is sufficiently induced by NE.

Cold-Induced Increase in Food Intake

Food intake increases with cold exposure (Donhoffer & Vonotzky, 1947; Weiss, 1958) and hypothalamic NE plays a role in food intake (Booth, 1968). In the present results, food intake in the water-drinking group and NE level in the hypothalamus were significantly higher in the cold than at normal temperature. Thus hypothalamic NE may contribute to enhanced eating behavior in cold. The reason for higher food intake during cold exposure in water-drinking than in saline-drinking mice is not clear.

There remains the question why, if cold exposure enhances PRA as suggested above, PRA is not activated equally in groups drinking water and saline. It may be relevant that, during 6-h cold exposure, potassium intake from food in the water group (11·2 mg) was about twice that in the saline group (6·1 mg). Increased plasma potassium inhibits renin release and PRA by increasing delivery of sodium to macula densa in kidney (Flamenbaum *et al*., 1973; Sealey *et al*., 1970; Vander, 1970). Therefore, there is a possibility that high potassium intake in the water group suppressed PRA in the cold. Thus, the present results suggest that food intake which is increased during cold exposure not only induces the dietary induced thermogenesis and provides extra energy (Macari *et al*., 1983) and salt but also plays a role in PRA suppression.

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