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Role of Dietary Sodium and Chloride in Salt Appetite of Wistar Rats

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ABSTRACT

The relative importance of sodium and chloride in the genesis of salt appetite is not known. We therefore studied the effect of dietary regimens deficient either in sodium chloride (NaCl), or in sodium alone, or in chloride alone, on saline preference of normal Wistar rats. The animals received first a diet with a normal NaCl content (1%) for 15 days. They were then randomly allocated to 3 groups receiving a low NaCl diet, a Na-deficient diet, and a Cl-deficient diet, respectively. Before and after 19 days on such diets, salt appetite was tested using the two-bottle choice test, one bottle containing water and the other 0.3 M saline. Rats submitted to either NaCl or Na deprivation had a significant increase in salt appetite ($P<0.01$), whereas rats on Cl-depletion had not. No changes were observed for water intake, systolic blood pressure, plasma Na or blood pH. Plasma Cl and K decreased on the Cl-deficient diet ($P<0.05$). Plasma renin activity and urinary aldosterone excretion were increased in NaCl- and Na-depleted rats compared with Cl-depleted animals ($P<0.05$). In conclusion, salt appetite in rats is stimulated by Na-depletion, but not by chloride depletion. A relation with the renin–angiotensin–aldosterone system is probable, but other mechanisms may also be involved.

INTRODUCTION

During recent years, the role of dietary sodium chloride (NaCl) in the pathogenesis, the severity, and the maintenance of arterial hypertension has been the subject of numerous and sometimes passionate debates. The relation between salt intake and blood pressure is much more complex than was assumed during the sixties and seventies.

Interestingly, in salt-sensitive rats (Kotchen et al., 1983) and salt-sensitive hypertensive patients (Kurtz et al., 1987), the induction of an elevation of blood pressure in response to NaCl requires sodium and chloride to be administered together. Equimolar sodium loading without chloride had lesser effects on pressure (Whitescarver et al., 1984; Kurtz et al., 1987). Similarly, selective dietary chloride loading without sodium had no pressor effects in humans or in most animal models (Grollman et al., 1945; Whitescarver et al., 1984). The administration of sodium citrate or sodium bicarbonate led to either no change (Kurtz et al., 1987) or even a decrease (Luft et al., 1990) in blood pressure compared with NaCl.

During our investigations on the relation between salt consumption and blood pressure regulation, we have been interested to learn more about the stimuli underlying salt appetite. These interrelations may be of interest as salt appetite, thirst, and blood pressure all appear to be regulated in similar brain structures (Weiss et al., 1986). In addition, various treatments such as central converting enzyme blockade (DiNicolantonio et al., 1982), peripheral DOCA administration (Hamlin et al., 1988), and psychological stress (Ely et al., 1987) have concurrent influences on salt appetite and blood pressure.

We therefore undertook a first study (Drüeke and Muntzel, 1991) in adult Fischer 344 and Wistar rats because the former are remarkable for their relative lack of salt appetite compared with the latter when switched from a normal NaCl diet to a NaCl-free regime (Midkiff et al., 1987). However, no studies have examined the possible physiological basis of this behavorial abnormality. We examined salt appetite, renal electrolyte balance, plasma volume, plasma renin activity, and systolic blood pressure in rats consuming first normal (1% wt/vol) NaCl and subsequently a NaCl-free (0%) diet for 12 days each. We found that salt appetite was increased in Wistar rats following a period of NaCl deficiency, but was absent in Fischer 344 rats. In comparison to Wistar rats, Fischer 344 rats were further characterized by a high renin status, which was not sensitive to

TABLE 1

Intakes of distilled water and 0.3 M saline during 24-h two-bottle choice tests

	Low NaCl		Low Na		Low Cl	
	Control	Deficient	Control	Deficient	Control	Deficient
Water intake (ml/24 h)	29.3±3.2	27.2±2.9	26.3±2.5	27.8±3.3	30.0±2.5	31.1±2.4
Saline intake (ml/24 h)	20.1±3.5	55.5±6.1*	19.0±4.9	57.8±8.9*	19.7±2.1	22.5±4.5

Values are means±SE; n = 11 rats/group. *$p<0.01$ relative to control condition.

changes in dietary salt. However, these alterations did not affect systolic blood pressure or urinary electrolyte excretion. Altered renin metabolism in Fischer 344 rats, either peripheral or central, may be partly responsible for the relative lack of salt appetite in this strain and could be related to their notable lack of blood pressure sensitivity to dietary NaCl (Hall et al., 1976).

In the present study, we asked whether there were selective effects of dietary sodium and chloride on salt appetite since this issue has not been addressed previously. We therefore examined the influence of a dietary depletion of either sodium and chloride, of sodium but not of chloride, or of chloride but not of sodium on the development of salt appetite in Wistar rats.

MATERIAL AND METHODS

Eight-week-old male Wistar rats of a body weight between 250 and 340 g were housed in a temperature-controlled room and submitted to a 12 h/12 h day/night cycles. Unless indicated the animals had access to distilled water ad libitum. Initially, rats consumed a control diet containing 1.0% NaCl, 1.5% $CaCO_3$, 0.8% KCl, and 1.1% KH_2PO_4. After 2 weeks, the rats were divided into 3 groups. The first received a low-NaCl diet (low NaCl), the second an equimolar chloride-replaced, sodium-deficient diet (low Na), and the third an equimolar sodium-replaced, chloride-deficient diet (low Cl). The low NaCl diet was identical to the original diet, except NaCl was omitted and 0.8% KCl was replaced by 0.8% potassium lactate. The chloride-depleted and the sodium depleted diets were obtained according to Whitescarver et al. (1984 and 1986). All diets contained adequate amounts of sucrose, casein, corn oil, minerals, and vitamins.

Salt appetite was evaluated using a two-bottle choice test, first on the final day of the 1% NaCl control diet and then after 19 days on the deficient diets. Rats were housed in individual cages and had access to 2 graduated cylinders equipped with water spouts, one filled with 0.3 M saline and the other with distilled water. Both cylinders were placed on the home cage at the beginning of the dark cycle, and their positions alternated from rat to rat to avoid position preference. Saline and water consumption were measured gravimetrically after 24 h. Systolic blood pressure (SBP) was recorded by the tail cuff method in awake rats using a programmed electrosphygmomanometer on the 8th day of each dietary regime. Urinary output, aldosterone, and electrolyte excretion were determined on day 12 of control and deficient diet periods. Blood was drawn at day 13 for plasma electrolytes and extracellular fluid volume status, and at day 14 for the determination of acid-base status and plasma renin activity (PRA). Electrolytes, creatinine, urea, total protein, blood gases, and pH were measured according to standard methods, and aldosterone (Corvol et al., 1977) and PRA (Ménard and Catt, 1972) using radioimmunoassays.

Statistical analysis was done using appropriate single- or repeated-measures ANOVA, and Student–Newman–Keuls test for differences between independently obtained means.

RESULTS

Experimental animal groups were composed of 11 rats each. All groups showed moderate and identical saline intakes while consuming the 1% NaCl control diet. Rat groups consuming the low-NaCl diet and sodium-deficient diets nearly tripled saline intake, whereas salt appetite in chloride-deficient rats did not change (Table 1). A two (repeated measure) by three (diet) ANOVA showed a significant interaction ($P<0.001$) reflecting enhanced saline intakes only in the low-NaCl and sodium-deficient rat groups.

Voluntary water intake was not influenced by dietary regimen. There was no significant difference in body weights at the start and the end of experiments (data not shown). Food consumption increased, and daily water consumption decreased in

TABLE 2

Systolic blood pressure (SBP), plasma renin activity (PRA), and plasma biochemistry of rats fed control and deficient diets

	Low NaCl		Low Na^+		Low Cl^-	
	Control	Deficient	Control	Deficient	Control	Deficient
SBP (mm Hg)	135±2.6	135±4.7	135±3.0	135±3.4	139±3.5	140±4.5
PRA ($ng \cdot ml^{-1} \cdot h^{-1}$)	N.D.	31.3±3.2	N.D.	34.9±5.3	N.D.	18.7±3.3*
PNa^+ (mmol/l)	145±2.1	143±1.3	144±1.7	141±0.4	145±1.4	143±0.5
PCl^- (mmol/l)	104±1.8	101±1.3	105±1.4	104±0.4	104±1.4	97±0.9*
PK^+ (mmol/l)	N.D.	3.4±0.2	N.D.	3.9±0.3°	N.D.	3.3±0.01

Values are means±SE. P, plasma. *$p<0.05$ relative to other groups in deficient condition; °$p<0.05$ relative to low-Cl^- group in deficient condition. N.D., not determined.

all rats during the time period of deficient diets but these changes were comparable in all groups (data not shown).

Mean SBP was 136.0 mm Hg during the 1% NaCl diet and 136.8 mm Hg after 8 days on deficient diets. It did not differ between groups (Table 2). Creatinine clearance remained unchanged throughout, except in the rat group subjected to the chloride-deficient diet where it increased significantly (data not shown).

Changes of plasma electrolytes are shown in Table 2. Plasma sodium tended to decrease in all groups during sodium deficiency but this change was not significant ($P<0.07$). Plasma chloride, however, decreased significantly ($P<0.001$) during deficient diets, with the greatest decline on the chloride-deficient diet. Plasma potassium was higher in sodium-deficient rats compared with chloride-deficient rats ($P<0.05$). Plasma total protein, urea, and creatinine increased slightly and similarly in all groups during the deficient diets compared with the control period ($P<0.05$, data not shown). Plasma total protein was higher on NaCl-deficient diet compared with the two other deficient diets (72.3±0.7 vs. 69.3±1.1 and 68.9±0.4 g/l, $P<0.01$). PRA, which was measured only during the deficiency phase, was lower in the chloride-deficient group than in the other two conditions.

Urinary electrolyte excretions were influenced by dietary sodium and chloride deprivation in the expected directions. Urinary sodium decreased to negligible amounts in the low-NaCl group (from 2.6±0.1 [control] to 0.1±0.0 mmol/24 h, means±SEM, $P<0.001$) and the sodium-deficient group (from 2.3±0.3 [control] to 0.2±0.0 mmol/24 h, $P<0.001$) but remained the same in the chloride-deficient diet (2.3±0.2 [control] vs. 2.7±0.1 mmol/24 h, P = NS). Urinary chloride excretion was also identical in all groups during control period and declined in the low-NaCl group (from 4.4±0.2 [control] to 0.2±0.1 mmol/24 h, $P<0.001$) and the chloride-deficient group (from 4.1±0.3 [control] to 0.3±0.1 mmol/24 h, $P<0.001$) whereas it declined much less in sodium-deficient diet (from 4.2±0.6 [control] to 2.7±0.2 mmol/24 h, $P<0.05$ relative to other groups in deficient condition). Daily urinary potassium excretion was not influenced by the diets (data not shown). Urinary aldosterone excretion, while similar during control periods, remained stable on the chloride-deficient (7.8±0.9 ng/24 h) diet but increased markedly ($P<0.001$) in the other two groups (228±30 [NaCl-deficient diet] and 440±118 ng/24 h [Na-deficient diet]).

Finally, low-NaCl and sodium-deficient rats had lower plasma bicarbonate concentrations (20.6±0.8 and 20.1±0.6 mM) than chloride-deficient rats (24.3±0.7 mM, $P<0.05$). A similar difference existed for PCO_2 which was lower in the former two groups than in the latter (data not shown). Blood pH, however, was not different among groups (data not shown).

CONCLUSIONS

The main finding of the present study in normal rats is the observation that sodium deficiency, but not chloride deficiency, is responsible for the generation of saline preference and hence salt appetite. This finding is in keeping with the previously reported preference of salt-deprived animals for sodium-containing salts, compared with salts containing cations other than sodium (Bell and Sly, 1977).

Although it is tempting to speculate that elevated renin and aldosterone were responsible for the stimulation of salt appetite, it has not always been easy to link peripheral RAA activity to changes in voluntary salt intake. Systemic administration of renin (Chiaraviglio, 1976), angiotensin II (Findlay and Epstein, 1980), aldosterone (Weisinger and Woods, 1971), and DOCA (Zhang et al., 1984) have all been shown to stimulate salt appetite. However, reports have been conflicting (Fitzsimons and Wirth,

1978), and pharmacological doses together with prolonged treatments were necessary. The intracerebral administration of renin (Avrith and Fitzsimons, 1980) and angiotensin II (Zhang et al., 1984) stimulated salt appetite in rats whereas converting enzyme inhibition and angiotensin II receptor blockade suppressed salt intake (Weiss et al., 1986). In the rats of the present study, it is possible that higher renin and aldosterone levels were somehow related to the enhanced salt appetite when the animals consumed the NaCl- and sodium-deficient diets. A direct relationship was unlikely, however, because aldosterone was highest in sodium-deficient rats, whereas sodium appetite in this group was not greater than in the NaCl-depleted group.

PRA was increased on the NaCl- and sodium-deficient diets whereas it was unchanged on the chloride-deficient diet. A lack of PRA suppression on the sodium-deficient, but chloride-sufficient, diet was unexpected because supplemental chloride has been previously shown to suppress PRA in Sprague–Dawley rats (Kotchen et al., 1983 ; Ott et al., 1989). This difference may be due to differences in strains or other experimental conditions.

The differences in salt appetite may also be secondary to central or hepatic mechanisms, for instance via changes of angiotensin II receptor density in discrete brain regions (Moe, 1987) or putative sodium sensitive receptors in the liver (Tordoff et al., 1986).

In summary, the stimulation of salt appetite by dietary sodium-chloride deprivation depends on a selective deficiency of sodium in the diet, but not on that of chloride. The complex mechanisms which play a role in the control of salt appetite clearly require further research efforts.

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