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Renin, Water Drinking, Salt Preference and Blood Pressure in Alcohol Preferring and Alcohol Avoiding Rats

JAAKKO LINKOLA,[1] ILKKA TIKKANEN,[1] FREJ FYHRQUIST[1] AND MAIJA RUSI[2]

The Minerva Institute for Medical Research,[1] Box 819, SF-00101 Helsinki 10, Finland
and
The Research Laboratories of the State Alcohol Monopoly (Alko),[2] Box 350, SF-00101 Helsinki 10, Finland

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LINKOLA, J., I. TIKKANEN, F. FYHRQUIST AND M. RUSI. *Renin, water drinking, salt preference and blood pressure in alcohol preferring and alcohol avoiding rats.* PHARMAC. BIOCHEM. BEHAV. 12(2) 293–296, 1980.—Mechanisms controlling fluid volume were studied in alcohol preferring AA (Alko, Alcohol) and alcohol avoiding ANA (Alko, Non-Alcohol) rats. Hypertonic sodium chloride solution (5%) given orally caused a higher dipsogenic response in AA rats than in the ANA's. One hour after ethanol loading (4.8 g/kg, by stomach tube), plasma renin activity of AA rats was four times as high as in ANA rats. ANA rats had higher degree of sodium chloride (0.9%) preference and higher blood pressure. The strain differences in voluntary salt intake and salt metabolism may modulate the consumption of calories and water as well as blood pressure and different reactivity of the renin system in AA and ANA rats.

Alcohol preference Blood pressure Drinking Ethanol Fluid volume control Hypertension Rat strains Renin

NUTRITIONAL factors appear to regulate voluntary ethanol drinking [18, 21, 24]. In support of this, studies on selected, outbred ethanol preferring (AA, i.e., Alko, Alcohol) and ethanol avoiding (ANA, i.e., Alko, Non-Alcohol) [8] rats have shown AA rats to have a higher caloric intake than the ANA's [9]. Earlier attempts to tie water metabolism to voluntary alcohol drinking have been unsuccessful [3, 7, 23]. We have compared the mechanisms controlling fluid volume in AA and ANA rats. Strain differences have been previously observed in ethanol diuresis, urinary sodium and arginine vasopressin excretion [14] as well as in plasma aldosterone and electrolyte concentration [15]. In this study we measured normal water drinking, salt arousal of drinking [1], plasma renin activity (PRA) and saline preference of AA and ANA rats. Because a recent report [26] indicates higher ethanol drinking in a hypertensive rat strain than in a hypotensive one, we also tested blood pressure of AA and ANA rats.

METHOD

AA and ANA rats (aged 4–6 months) of the F_{24}, F_{28} and F_{29} generation were used in these experiments. They had been tested 3–4 weeks earlier for their voluntary ethanol drinking [8]. Habituation and other environmental conditions were as described [8,9]. Standard rat foods: ASTRA-EWOS R-3 (Södertälje, Sweden) (FOOD I) and Alko-food (FOOD II) [9], having different salt composition (Table 1) were given. Drinking and eating were measured in individual cages.

FOOD I and tap water were given freely when spontaneous eating and drinking of male rats was measured, and until the administration of 5% NaCl solution (30 ml/kg, by stomach tube, at noon) into 10 female and 10 male AA and ANA rats to induce salt arousal of drinking. After the salt administration, drinking of tap water was measured during 3 hr with no access of food.

Voluntary saline (0.9% NaCl) drinking of 10 male AA and ANA rats was measured with a two-bottle choice method giving tap water as another source of drink. Before and during the first 3 weeks of this experiment, the rats were on FOOD I. During the latter 3 weeks the rats were on FOOD II.

PRA and blood pressure were determined before and 1 hr after the administration of 4.8 g/kg ethanol (20% v/v) by a stomach tube. Blood was collected from decapitated rats and PRA was measured by radioimmunoassay [11]. This method was modified for rats by using hydroxyquinoline (5.0 mM) as enzyme inhibitor and pH 6.5 in the angiotensin I generation step. Blood pressure was measured by a tail-cuff method [4] keeping the rats in an enclosed plywood box and leaving the tail free. The tail was first warmed for 10 min at 37°C. For statistical treatments Student's *t*-test was used.

RESULTS

AA rats ate more FOOD I and drank more tap water than ANA rats during 3 weeks (Table 2). When water drinking is calculated per g of eaten food, the strain difference declines.

TABLE 1

THE MAIN MINERAL COMPONENTS AND CALORIES OF FOOD I AND FOOD II

Mineral component	Food I	Food II
Sodium	2.4 g/kg	4.8 g/kg
Potassium	5.3 g/kg	2.2 g/kg
Magnesium	1.09 g/kg	0.90 g/kg
Calcium	1.5 %	0.20 %
Phosphorus	1.1 %	0.45 %
Useful calories	3.20 Mcal/kg	3.69 Mcal/kg

TABLE 2

SPONTANEOUS WATER DRINKING AND EATING OF MALE AA AND ANA RATS

Group	Water drinking ml/kg·day	Eating g/kg·day
AA (10)	116 ± 12*	72 ± 5*
ANA (9)	71 ± 28	52 ± 7

Values are expressed as means ± SD with the number of animals/group in parentheses.

*Significantly different from ANA group ($p<0.001$, Student's t-test).

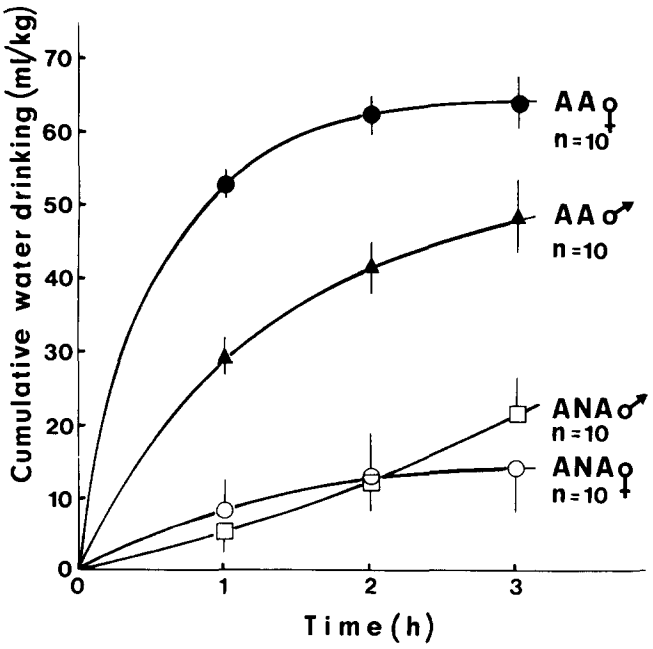

FIG. 1. Cumulative water drinking of AA and ANA rats after 5% NaCl solution given by stomach tube. Values are shown as mean ± SE.

AA rats displayed a more powerful dipsogenic response than ANA rats to osmotic stimulation with NaCl (Fig. 1). The most marked drinking response was in AA females. Strain difference was significant at each hour ($p<0.001$). Sex difference was significant in AA rats ($p<0.001$ at 1 and 2 hours and $p<0.025$ at 3 hours).

AA rats showed a more vigorously increased PRA after ethanol administration (Table 3). ANA rats, on the other hand, had higher blood pressure (Table 3) and a higher degree of saline preference, particularly on FOOD I (Fig. 2 and Table 4). Both rat strains preferred saline to water. Decrease in water drinking was compensated by increased saline drinking and vice versa. There was no marked strain differences in food intake, when saline was present. On FOOD II, both rat strains lowered their saline drinking. Strain differences in saline drinking, water drinking and body weight were more pronounced on FOOD I than on FOOD II.

DISCUSSION

AA and ANA rats displayed marked strain differences in the mechanisms controlling fluid volume. The results are thus compatible with previous data [14,15]. Spontaneous water drinking of AA and ANA rats correlated positively to eating (Table 2). However, AA rats drank at a faster rate water after hypertonic NaCl solution (Fig. 1). Thus, acutely stimulated thirst appeared more intense in AA than ANA rats. Interestingly, the most marked drinking response was in AA females, who previously [8] had the highest intake of alcohol and calories.

Renin release appeared to be more sensitive to stimulation with ethanol in AA than ANA rats (Table 3), but without ethanol the strain difference was not significant. This indicates that renin-angiotensin system could hardly alone explain water drinking of AA rats. However, the central ac-

TABLE 3

RENIN (PRA) AND SYSTOLIC BLOOD PRESSURE OF AA AND ANA RATS BEFORE AND ONE HOUR AFTER ETHANOL ADMINISTRATION

Group	PRA (ng/ml·hr) Before ethanol	PRA (ng/ml·hr) After ethanol	Blood pressure (mm Hg) Before ethanol	Blood pressure (mm Hg) After ethanol
AA	6.81 ± 2.08 (13)	32.04 ± 29.74 (20)*	126 ± 16 (11)†	114 ± 18 (11)†
ANA	5.35 ± 2.02 (12)	8.08 ± 4.04 (20)	146 ± 20 (12)	140 ± 30 (12)

Values are expressed as means ± SD with the number of animals/group in parentheses.

* or † Significantly different from ANA group ($p<0.001$ or 0.02, respectively by Student's t-test).

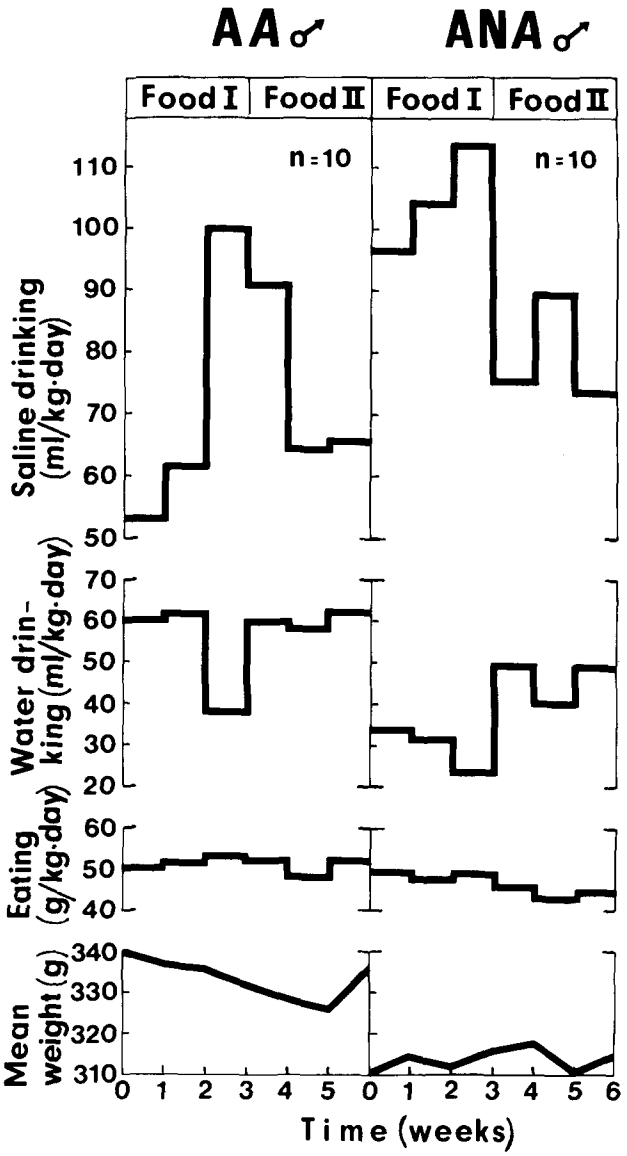

FIG. 2. Mean spontaneous saline and water drinking as well as simultaneous eating and weight of AA and ANA rats on FOOD I (weeks 1–3) and on FOOD II (Weeks 4–6).

tions of angiotensin II may be different in AA and ANA rats. This may be suggested on the basis of the following findings: (1) a synergism between Na ions and angiotensin II [2], (2) the lower inhibition of cerebral Na transport by ethanol in AA than in ANA rats [17], and (3) the higher Na conservation in the kidneys of AA rats than of the ANA's [14]. Thus, Na ions and angiotensin II might act synergistically to increase water drinking in AA rats, particularly at high PRA levels after ethanol. The stimulated renin-angiotensin system should also be considered a possible dipsogenic factor promoting ethanol drinking in AA rats.

Some potential explanations for higher PRA in AA rats may be offered. Higher PRA (Table 3) and prolonged tachycardia [13] in AA rats after ethanol administration may indicate higher sympathetic nervous activity in the ethanol preferring strain. Lower degree of saline preference and

TABLE 4

TOTAL SODIUM INTAKE DURING THE MEASUREMENT OF SALINE PREFERENCE (SEE FIG. 2)

Food	Week	Na intake mg/kg·day	Na taken in saline %
AA rats (10)			
	1	308.7 ± 146.0†	60.7
Food I	2	341.5 ± 142.7*	63.8
	3	481.1 ± 152.6	61.7
	1	572.8 ± 200.0	56.0
Food II	2	459.2 ± 157.5	49.4
	3	483.1 ± 138.4	47.9
ANA rats (10)			
	1	459.7 ± 113.5	74.0
Food I	2	482.7 ± 118.2	76.2
	3	519.9 ± 144.2	77.2
	1	486.2 ± 143.4	54.7
Food II	2	520.3 ± 188.9	60.6
	3	474.5 ± 216.0	54.5

Values are expressed as means ± SD with the number of animals/group in parentheses.

* or †Significantly different from ANA group ($p<0.05$ or 0.02, respectively by Student's t-test).

lower blood pressure in AA rats may contribute to their higher PRA response [5] to ethanol. It is noteworthy that angiotensin II stimulates catecholamine release from adrenals and sympathetic nerve endings and also directly causes tachycardia in cats, rabbits and greyhounds [20].

Both AA and ANA rats preferred saline to water (Fig. 2), which supports previous findings in rats [22,25]. The lesser capacity of ANA rats than the AA's for retaining Na [14] could account for higher saline intake (Fig. 2) and also for higher total Na intake (Table 4) of ANA rats. However, the detailed mechanisms of saline preference in ANA rats remain unclear.

The available food and drink may also influence saline preference. Thus, the rats are able to regulate their NaCl intake by way of NaCl in solution [10] or in food [12]. This may explain why ANA rats had higher saline drinking during the first three weeks while on low-Na FOOD I. This Na seeking effect may also cause the decrease of saline drinking and Na intake by way of saline (Table 4), when FOOD I was replaced by Na-rich FOOD II. Our results suggest that regulation of NaCl intake in rats may be determined both by NaCl in solution as well as in food.

The present data put emphasis on the relations between food intake, salt metabolism and ethanol preference of AA and ANA rats. Thus, when the rats were given free access to saline, the strain difference in total food intake (Table 2) declined (Fig. 2). This may be due to the inhibiting effect of NaCl on feeding mechanisms [19] in AA rats. Higher renal Na conservation capacity of AA rats may have amplified the effects of Na in these rats. The effects of NaCl on eating [22] as well as the Na seeking property of the rats [10,12] lead to a suggestion that Na avoidance may promote the preference of Na-free energy sources and contribute to ethanol drinking of AA rats. Likewise, high Na preference of ANA rats may lead them to seek salt containing energy sources. Thus, it

could be hypothesized the salt access combined with the properties of salt metabolism may significantly modify ethanol drinking at least in rats.

Blood pressure of ANA rats (Table 3) was higher than the normal systolic blood pressure values in rats reported by Dunn and Tannen [6] and also observed by us in Wistar and Sprague-Dawley rats (unpublished observations), while AA rats had a normal blood pressure. Na and water metabolism as well as strain differences in the central nervous system may partly explain the difference in blood pressure. Wood *et al.* [26] reported that voluntary ethanol intake was not causally related to blood pressure phenotype, although their hypertensive mice consumed more ethanol than the hypotensive ones. We were unable to tie high blood pressure to high voluntary ethanol drinking at all. On the contrary, high blood pressure appeared rather to be associated with ethanol avoidance. In conclusion, different thirst mechanisms in AA and ANA rats are suggested by this study. Moreover, different salt metabolism could partly account for strain differences in drinking behaviour, renin release, blood pressure and energy metabolism.

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