Relationship between dietary Na^+ intake, aldosterone and colonic amiloride-sensitive Na^+ transport

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The effect of changes in dietary Na^+ intake on plasma aldosterone levels and electrogenic amiloride-sensitive Na^+ transport (I_{amil}) was studied in the rat distal colon. Five groups of rats were fed on diets containing different amounts of Na^+. Estimated Na^+ intake ranged from about 400–80000 μequiv Na^+/kg body weight (BW) per d. Both variables investigated, I_{amil} and plasma aldosterone, depended non-linearly on Na^+ intake. Reduction of the daily Na^+ intake increased plasma aldosterone levels and if these levels reached the value 200 pg/ml or more then I_{amil} was induced. The corresponding Na^+ intake was 1300 μequiv Na^+/kg BW per d. I_{amil} was not observed at lower aldosterone levels and higher Na^+ intakes. Aldosterone infusion for 7 d produced similar changes in I_{amil} compared with dietary Na^+-depleted animals and made the estimation of maximum transport capacity of I_{amil} possible. We conclude that I_{amil} operates only if Na^+ intake decreases below minimal Na^+ requirement in growing rats and that the maximum transport capacity of this pathway is reached only after very severe Na^+ deprivation.

Sodium: Aldosterone: Colon

The colon is the terminal place for the regulation of intestinal output of ions and water. It represents the part of the intestinal tract which has the highest ability to conserve Na^+, i.e. to absorb Na^+ against high Na^+ concentration differences. It is now well established that Na^+ transport is not homogeneous throughout the colon and that there exist significant segmental differences (Binder *et al.* 1991). Rats adapted to a low-salt diet have an electrogenic amiloride-sensitive Na^+ transport system in the distal colon (Will *et al.* 1980; Edmonds, 1981; Halevy *et al.* 1986), whereas control rats transport Na^+ via an electroneutral Na–Cl pathway (Foster *et al.* 1983; Edmonds & Mackenzie, 1984). The suppression of this electroneutral pathway and the induction of electrogenic Na^+ transport is regulated by aldosterone (Perrone & Jenks, 1984; Halevy *et al.* 1986).

In contrast to adulthood, the colonic Na^+ transport process in rats is electrogenic and amiloride-sensitive during early postnatal life and this transport pathway disappears after weaning (Pácha *et al.* 1987, 1988). Since adult rats fed on a standard rat chow do not maintain the electrogenic amiloride-sensitive Na^+ transport, though the activity of this transport pathway is high in the distal colon of weanling rats on the same diet, the findings indicate that the electrogenic pathway might operate only during acute or chronic Na^+ deficiency of the organism. The Na^+ requirement for the growing rat is 0·5 g/kg ration (Warner & Breuer, 1978) but most commercially available standard diets have Na^+ in the amount of 3–7 g/kg (Aviv *et al.* 1982). As dietary Na^+ restriction has a direct stimulatory effect on plasma aldosterone levels and Na^+ surplus has the opposite effect (Douglas *et al.* 1978), it is obvious that dietary Na^+ intake has a direct influence on the induction and transport rate of Na^+ transport. An interesting question is which Na^+ intakes are recognized by the rat as 'low' or 'high' and what is the threshold level of Na^+ intake that inhibits Na–Cl electroneutral transport and induces the electrogenic Na^+ pathway.

The objectives of the present study, therefore, were (1) to determine how the changes in Na^+ supply influence the electrogenic amiloride-sensitive Na^+ transport and plasma levels of aldosterone, (2) to assess the threshold level of Na^+ intake for inducing the electrogenic Na^+ pathway and (3) to establish whether this transport pathway operates only if Na^+ intake is lower than the Na^+ requirement for growing rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (Institute of Physiology, Prague, Czech Republic) weighing 250–280 g were fed on a standard diet and given distilled water *ad lib*. The rats were caged individually at 21° having a 12 h light–dark cycle. At 1 week before the experiments the rats were divided into five groups and were allowed free access to feed containing different amounts of Na^+. Group 1 was fed on a Na^+-deficient rat chow from ICN Biochemicals (Cleveland, OH, USA) No. 902902, group 2 received a Na^+-deficient diet from Altromin (Lage, Germany) No. C1036, group 3 was fed on a low-salt diet, group 4 a standard diet and group 5 a high-salt diet from a local supplier (Table 1). These last three diets contained natural ingredients and either no NaCl or a small or a high addition of NaCl respectively. The low- and high-salt diets were the ones usually used in the studies of rat salt hypertension. The diets given to groups 1 and 2 were semi-purified diets prepared from relatively pure sources of carbohydrate, vegetable oils, purified casein, non-nutritive bulk and a vitamin and mineral mix (for details see ICN Biochemicals and Altromin International Catalogues). The Na^+ and K^+ contents of these diets were measured and the ionic content is given in Table 2. Feed consumption was measured daily and the corresponding Na^+ and K^+ intakes were calculated. To test whether dietary factors other than Na^+ intake are able to influence the variables investigated, some rats in groups 1, 2 and 3 received saline (9 g NaCl/l) instead of distilled water.

In some experiments a different method was used for raising plasma aldosterone levels and for inducing amiloride-sensitive Na^+ transport. The rats maintained on a standard diet and distilled water received a continuous infusion of aldosterone via subcutaneously implanted Alzet minipumps (Alza Corporation, Palo Alto, CA, USA) for 7 d. Aldosterone was dissolved in polyethylene glycol 400 and loaded into the minipumps. Hormone doses were normalized according to the animal's weight and the aldosterone was infused at rates of 200, 600 and 800 μg/kg body weight (BW) per d. These values were two, six or eight times higher than the *in vivo* values of adrenal secretion rates of aldosterone into adrenal venous blood of Na^+-depleted rats (Vinson *et al.* 1985).

At the beginning of the experiment the rats were anaesthetized with ether, blood was withdrawn from the abdominal aorta and the distal colon was removed for assessment of Na^+ transport.

Transport measurements

Electrogenic amiloride-sensitive Na^+ transport was assessed by measuring amiloride-sensitive short-circuit current (I_{amil}). The distal colon was rinsed carefully to remove the lumen contents, stripped of the serosa and part of the muscle layers and mounted in an Ussing chamber. The epithelium was bathed on both sides with a modified Ringer solution. The composition of the solution was (mmol/l): Na^+ 140·0, K^+ 5·4, Ca^{2+} 1·2, Mg^{2+} 1·2, Cl^- 123·8, HPO_4^{2-} 2·4, $H_2PO_4^-$ 0·6, HCO_3^- 21·0, glucose 10·0, β-hydroxybutyrate 0·5, glutamine 2·5 and mannitol 10·0; pH 7·4 at 37° and oxygenation with O_2–CO_2 (95:5 v/v). The tissues were short-circuited to zero as described previously using an automatic voltage-clamp system which was corrected for fluid resistance and for potential asymmetry of the

Table 1. *Composition of the non-purified diets (g/kg diet)*

Diet...	3	4	5
Wheat	600	600	600
Casein	130	130	130
Milk powder	130	130	130
Bran	90	90	10
Vitamin and salt mix	50*	50**	50**
NaCl	—	—**	80**

* NaCl-free salt and vitamin mixture contained (g/kg mixture): $CaCO_3$ 84·43, $CaHPO_4$ 220·70, $FeSO_4$ 31·95, $CuSO_4$ 0·392, $ZnSO_4$ 0·44, $MnCO_3$ 1·88, KI 0·026, choline chloride 20·0, inositol 2·0, thiamine hydrochloride 0·240, riboflavin 0·160, pyridoxine hydrochloride 0·08, cyanocobalamin 0·0006, tocopherol acetate 2·0, pteroylglutamic acid 0·120, calcium pantothenate 0·160, retinol 0·03, ergocalciferol 0·006, dried yeast 400·0; the ingredients were triturated in dextrose.

** In diets 4 and 5, the standard vitamin and salt mixture (Farmakon, Olomouc, Czech Republic) was used. The composition was the same as in diet 3 except that dextrose was partly replaced by 150·0 g NaCl; extra NaCl was added to diet 5.

Table 2. *Na^+ and K^+ contents of the diets (μequiv/g diet)*

(Mean values with their standard errors for four analyses)

Group*...		1	2	3	4	5
Na^+	Mean	7	13	23	126	1599
	SE	0	1	2	9	109
K^+	Mean	266	155	213	235	228
	SE	7	4	6	7	6
K^+:Na^+		38·0	11·9	9·3	1·9	0·1

* For details, see p. 634.

electrodes (Pácha *et al.* 1987). Amiloride, a specific blocker of electrogenic amiloride-sensitive Na^+ transport (Benos, 1982), was added to the mucosal side of the tissue to reach a final concentration of 10^{-4} M.

Analysis of blood and feed

Blood was centrifuged (3000 rev./min for 20 min at 4°), the plasma was removed, transferred into appropriate tubes for the respective assay and frozen at −70°. Plasma aldosterone was measured by a commercial radioimmunoassay kit (Adico Ltd, Prague, Czech Republic) in 100 μl triplicate samples of the plasma. Plasma Na^+ and K^+ were measured by flame photometry, Cl^- by AgCl titration and osmolality by determining the depression of the freezing point. Feed samples were processed by shaking the pellets with concentrated HNO_3 for 48 h and Na^+ and K^+ concentrations were estimated in the supernatant fraction using flame photometry.

Statistical methods

Experimental values are reported as means with their standard errors. Further statistical analysis was performed using BMDP programs (Dixon, 1988). The differences among different dietary groups were analysed by one-way analysis of variance. When significance was found, the Bonferroni test was used for significant differences between pairs of groups. A 95% confidence level was used to define statistical significance.

Table 3. *Dietary intake of Na^+ and K^+ (μequiv/kg BW per d) by rats**
(Mean values with their standard errors for five to nine animals)

Group...		1	2	3	4	5
Na^+†	Mean	385	747	1339	6704	81570
	SE	17	32	74	316	4783
K^+	Mean	13570	8840	12350	12460	11630
	SE	1090	990	1110	870	820

* For details of diets and procedures, see Tables 1 and 2 and p. 634.

† Analysis of variance demonstrated significant differences among the groups ($P < 0{\cdot}001$) for Na^+ intake and the differences between all pairs were highly significant ($P < 0{\cdot}001$).

RESULTS

The mean values of Na^+ and K^+ intake over the 7 d feeding period are shown in Table 3. There was a 211-fold increase of Na^+ intake between group 1 and group 5 and the Na^+ intake in each group was statistically significantly different from all other groups. The K^+ content in all diets was approximately constant and hence the K^+ intake was similar in all the groups. In strident contrast to the Na^+ intake in all the various groups, Na^+ and K^+ concentrations in the arterial blood did not differ significantly (Table 4). Plasma Na^+ and K^+ concentrations of rats fed on high-Na^+ diets were not different from those of the control or Na^+-deficient rats. Plasma Cl^- concentration and osmolality were significantly changed in some groups but there was no correlation between these changes and dietary Na^+ intake. This means that the homeostatic mechanisms in the rat are able to compensate for very high or very low dietary Na^+ intake effectively.

Low Na^+ intake had a stimulatory effect on both plasma aldosterone and electrogenic amiloride-sensitive Na^+ transport. High Na^+ intake had the opposite effect. Fig. 1 demonstrates the correlation between Na^+ intake and plasma aldosterone or I_{amil} which was used for assessment of amiloride-sensitive Na^+ transport. The aldosterone concentration and I_{amil} behaved non-linearly with increasing Na^+ intake. The threshold Na^+ intake for the induction of I_{amil} in the distal colon was below 1300 and above 750 μequiv Na^+/kg BW per d. As the daily intake of the rats on the standard diet was 6700 μequiv Na^+/kg BW per d it is obvious that electrogenic amiloride-sensitive Na^+ transport cannot operate in the rat distal colon under standard conditions. The plasma aldosterone concentration which was able to induce measurable I_{amil} was more than 200 pg/ml.

To test whether some dietary factors other than Na^+ might also be involved in the large differences among groups 1, 2 and 3 we investigated the effect of salt repletion on plasma aldosterone and amiloride-sensitive Na^+ transport. If these rats drank saline instead of distilled water, no amiloride-sensitive Na^+ transport was induced and plasma aldosterone was not significantly different from that of control rats kept on standard diet (group 4); plasma aldosterone was only 161 (SE 23) pg/ml (n 5) in group 1, 138 (SE 19) pg/ml (n 4) in group 2 and 102 (SE 27) pg/ml (n 4) in group 3. The results demonstrate that the observed differences are solely due to the different Na^+ contents in the diets.

Additional experiments were performed in animals that received an infusion of aldosterone for 7 d to determine the maximum capacity of electrogenic amiloride-sensitive Na^+ transport. The effect of aldosterone on I_{amil} is shown in Table 5. The results demonstrate that the groups with higher infusion rates had higher plasma aldosterone levels which were not associated with greater I_{amil}. Fig. 2 summarizes the data obtained in the experiments with delivery of exogenous aldosterone and with low-salt diets and

Table 4. *Plasma electrolyte concentration (mmol/l) and osmolality (mosmol/kg H_2O) of rats kept on various dietary Na^+ intakes*

(Mean values with their standard errors for five to nine animals)

Group...		1	2	3	4	5
Na^+	Mean	145·7	146·4	147·7	145·5	147·9
	SE	1·4	1·0	0·8	1·1	1·9
K^+	Mean	4·2	4·0	4·1	4·1	4·4
	SE	0·1	0·1	0·2	0·2	0·4
Cl^-	Mean	96·5	99·6	104·4**	100·7	101·0
	SE	0·4	1·5	0·9	1·0	1·2
Osmolality	Mean	298	286**	290*	296††	292
	SE	2	1	2	1	2

Mean values were significantly different from those of group 1: * $P < 0·05$, ** $P < 0·01$.

†† Mean value was significantly different from that of group 2, $P < 0·01$.

Analysis of variance demonstrated no differences in plasma Na^+ or K^+ but the differences in plasma Cl^- and osmolality were highly significant ($P < 0·002$ and $P < 0·001$ respectively).

‡ For details of diets and procedures, see Tables 1 and 2 and pp. 634–635.

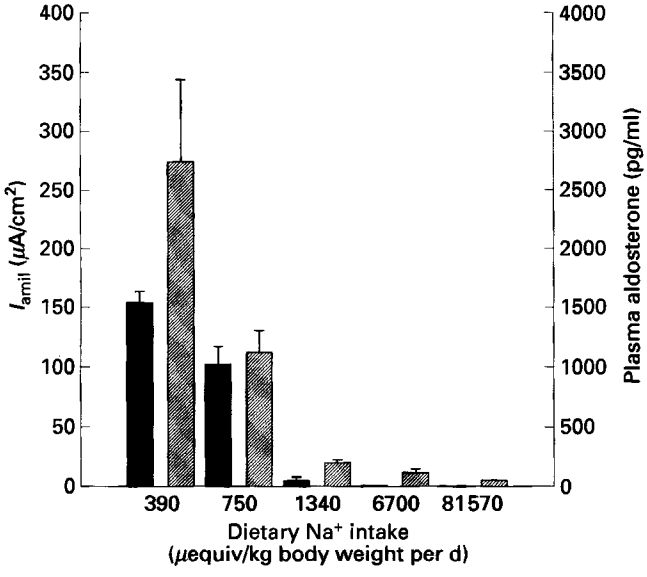

Fig. 1. Plasma level of aldosterone (▨, pg/ml) and electrogenic amiloride-sensitive Na^+ transport (I_{amil}, ■, $\mu A/cm^2$) plotted against dietary Na^+ intake (μequiv/kg body weight per d) in rats. Values are means with their standard errors represented by vertical bars (n 5–9). Analysis of variance demonstrated a significant effect of salt intake on plasma aldosterone and I_{amil} ($P < 0·001$). Significant differences for plasma amiloride: group 1 *v.* group 2, $P < 0·01$; group 1 *v.* group 3–5, $P < 0·001$; group 2 *v.* group 3–5, $P < 0·01$. Significant differences for plasma aldosterone levels: group 1 *v.* group 2, $P < 0·01$; group 1 *v.* group 3–5, $P < 0·001$; group 3 *v.* group 5, $P < 0·001$; group 4 *v.* group 5, $P < 0·01$.

Table 5. *Plasma aldosterone levels and electrogenic amiloride-sensitive Na^+ transport (I_{amil}) in rats during the delivery of aldosterone via osmotic minipumps**

(Mean values with their standard errors)

Infusion rate (μg aldosterone/kg BW per d)	*n*	Plasma aldosterone (pg/ml)		I_{amil} ($\mu A/cm^2$)	
		Mean	SE	Mean	SE
200	5	1374	360	134	15
600	4	4510	480	262	30
800	5	6055	705	251	27

* For details of procedures, see p. 634.

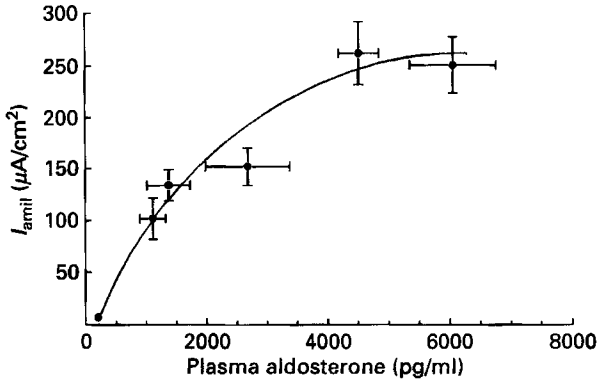

Fig. 2. Relationship between the rate of electrogenic amiloride-sensitive Na^+ transport (I_{amil}; $\mu A/cm^2$) in the rat distal colon and plasma aldosterone levels (pg/ml). The curve was drawn by eye.

illustrates the relationship between I_{amil} and plasma aldosterone. The data reveal that the maximum transport capacity of the amiloride-sensitive pathway is approximately 250 $\mu A/cm^2$.

DISCUSSION

Dietary Na^+ changes have frequently been used to study the effect of aldosterone on intestinal and renal epithelial transport (Edmonds, 1981; Edmonds & Mackenzie, 1984; Binder *et al.* 1991; Rossier & Palmer, 1992). However, it has not been shown which level of Na^+ intake is critical for the regulation of Na^+ transport by aldosterone and whether the transport changes grow through an abrupt or gradual process. It is obvious from the present study that dietary Na^+ deficiency markedly stimulates electrogenic amiloride-sensitive Na^+ transport in the rat distal colon. Our results show that the threshold Na^+ intake which is able to induce this transport pathway is below 1300 μequiv Na^+/kg BW per d and that the relationship between Na^+ intake and I_{amil} is non-linear. In the range of Na^+ intakes from 1300 to 750 μequiv Na^+/kg BW per d we can see practically total inhibition of I_{amil} (92%). The facts that we found I_{amil} only in rats fed on diets containing less than 24 μequiv Na^+/g diet and that standard commercially available diets including standard Purina (Foster *et al.* 1983), Altromin (Horster & Lückhof, 1983) and Wayne Blox (Aviv *et al.* 1982) rat chows contain 100–250 μequiv Na^+/g diet suggest that the standard level of Na^+ intake in rats is usually above the threshold level of 1300 μequiv Na^+/kg BW per d.

This is consistent with the Na^+ intake measured in rats fed on the standard diets purchased from various sources. These values are in the range 3300–11300 μ equiv Na^+/kg BW per d (Lundin *et al.* 1982; Cangiano *et al.* 1984; Lübcke & Barbezat, 1988; Nishi *et al.* 1992). This means that it is not possible to detect I_{amil} in rat distal colon, and probably also in other aldosterone target tissues, under normal conditions. A similar Na^+ restriction has a stimulating effect on electrogenic amiloride-sensitive Na^+ transport in cortical collecting tubules of the rat kidney (Pácha *et al.* 1993).

The Na^+ requirement of the rat is 0·5 g/kg ration (Warner & Breuer, 1978). The daily feed consumption of a rat corresponds to approximately 5% of its body mass so that a diet consisting of 0·5 g Na^+/kg would provide it with a daily Na^+ intake of 1090 μequiv Na^+/kg BW per d. Feed consumption in the present experiments was 51–64 g/kg BW per d and thus the critical Na^+ intake would be 1110–1390 μequiv Na^+/kg BW per d. Comparing the calculated critical values with Na^+ intakes in the groups which manifested I_{amil} (Fig. 1), our results strongly suggest that electrogenic amiloride-sensitive Na^+ transport is induced and operates only if the rat is subjected to a substantial degree of Na^+ deprivation.

Aldosterone has been implicated as the regulatory hormone of the electogenic amiloride-sensitive Na^+ transport in the rat colon as well as in other 'tight' epithelia (Will *et al.* 1980, 1985; Edmonds, 1981; Halevy *et al.* 1986). Our results confirm this conclusion and demonstrate a close relationship between plasma aldosterone and I_{amil}. It can be concluded that plasma aldosterone levels higher than 200 pg/ml are needed for inducing electrogenic amiloride-sensitive Na^+ transport. Although Na^+ deprivation elevates plasma aldosterone levels, it is possible that the changes in colonic Na^+ transport during Na^+ deprivation do not reflect the maximum capacity of the amiloride-sensitive Na^+ pathway. We therefore designed experiments to compare Na^+ transport in the distal colon of rats that had received continuous aldosterone infusion via implanted minipumps with the Na^+ transport of Na^+-depleted animals. Infusion of aldosterone resulted in plasma aldosterone levels that were comparable with or higher than those observed after dietary Na^+ deprivation. Based on these experiments, we can conclude that the maximum capacity of electrogenic amiloride-sensitive Na^+ transport is 250 $\mu A/cm^2$, i.e. 9·33 μequiv Na^+/h per cm^2. Assuming that the transport capacity is 152 $\mu A/cm^2$ if Na^+ intake is only 390 μequiv Na^+/kg BW per d, then the rats have a considerable ability to compensate for disturbances in dietary Na^+ intake.

Like the relationship between Na^+ intake and I_{amil}, the relationship between Na^+ intake and plasma aldosterone is also non-linear. It is evident from Fig. 1 that the twofold increase in Na^+ intake (1340 *v.* 750 μequiv Na^+/kg BW per d) is associated with an 82% inhibition of plasma aldosterone but a fivefold increase in Na^+ intake between 1340 and 6700 μequiv Na^+/kg BW per d is associated only with 35% inhibition. Plasma aldosterone levels depend not only on the Na^+ but also on the K^+ intake (Douglas *et al.* 1978). This means that the Na^+:K^+ intake ratio plays a decisive role in the regulation of plasma aldosterone. However, in the present experiments the range of Na^+ intake was 211-fold from group 1 to group 5 and only 1·5-fold in relation to the K^+ intake which did not vary in the individual groups in a systematic way. We therefore believe that the differences in K^+ intake cannot account for the observed changes in plasma aldosterone and I_{amil}.

In summary, these results suggest that the electrogenic amiloride-sensitive Na^+ transport and plasma aldosterone levels behave non-linearly with increasing Na^+ intake. This transport pathway is triggered in the rat distal colon if Na^+ intake declines below 1300 μequiv Na^+/kg BW per d and if the plasma aldosterone level is higher than 200 pg/ml. The maximum transport capacity seems to be reached only after very severe Na^+ deprivation. We conclude that the electrogenic amiloride-sensitive pathway operates only if the Na^+ intake decreases below the minimal Na^+ requirement for the growing rat.

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