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The Role of Chloride in Deoxycorticosterone Hypertension: Selective Sodium Loading by Diet or Drinking Fluid

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Summary

To evaluate the role of chloride in the pathogenesis of salt-dependent deoxycorticosterone (DOC) hypertension, we studied young Wistar rats chronically loaded with sodium bicarbonate ($NaHCO_3$) or sodium chloride (NaCl) which were administered either in the diet or in the drinking fluid. Selective sodium loading (without chloride) increased blood pressure (BP) in DOC-treated animals only if $NaHCO_3$ was provided in the diet. In contrast, no significant blood pressure changes were induced by DOC treatment in rats drinking $NaHCO_3$ solution. Hypernatremia and high plasma osmolality occurred only in rats drinking NaCl or $NaHCO_3$ solutions. Compared to great volume expansion in NaCl-loaded DOC-treated rats, the degree of extracellular fluid volume expansion (namely of its interstitial fraction) was substantially lower in both $NaHCO_3$-loaded groups in which significant hypokalemia was observed. $NaHCO_3$-drinking rats without significant blood pressure response to DOC treatment represented the only experimental group in which blood volume was not expanded. In conclusion, our data confirm previous observations that $NaHCO_3$ loading is less potent in eliciting DOC hypertension than NaCl loading, but blood pressure rise in rats fed $NaHCO_3$ diet clearly demonstrated that selective sodium loading could potentiate the development of DOC hypertension if $NaHCO_3$ is offered within the appropriate dietary regimen. The reasons for the failure of $NaHCO_3$-drinking rats to elevate blood pressure in response to chronic mineralocorticoid treatment are not obvious. However, the absence of a significant plasma volume expansion together with hypernatremia and increased plasma osmolality suggest a considerable degree of dehydration in these animals which fail to increase their fluid consumption compared to water drinking rats.

Key words
Sodium • Chloride • Bicarbonate • Blood pressure • Body fluids • Blood volume

Introduction

It has been reported that chronic selective sodium loading, without a concomitant increase of chloride intake, failed to induce hypertension in Dahl salt-sensitive rats (Kotchen *et al.* 1983, Whitescarver *et al.* 1984) as well as in DOC-treated rats (Kurtz and Morris 1983, 1985). Since dietary selective chloride loading was also insufficient to produce hypertension in Dahl rats (Whitescarver *et al.* 1986a, Reddy and Kotchen 1992) or DOC-treated rats (Passmore and Jimenez 1990, Imig *et al*. 1993, Kadota *et al.* 1993), it was concluded that both ions participate in the pathogenesis of hypertension caused by chronic sodium chloride overload in the rat (Blaustein 1985, Kurtz and Morris 1986, Whitescarver *et al.* 1986b). Selective sodium loading was

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achieved by either drinking of $NaHCO_3$ solution (Kurtz and Morris 1983, 1985) or feeding diets with sodium accompanied by anions other than chloride (Kotchen *et al.* 1983, Whitescarver *et al.* 1984, Passmore *et al.* 1985). Currently, there is no direct comparison of the influence exerted by these sodium salts when added to the drinking fluid or to the diet. Such comparison might be rather important because high dietary sodium intake without chloride produced a mild to moderate blood pressure elevation in DOC-treated rats (Passmore *et al.* 1985, Motoyama *et al.* 1987, 1988, Passmore and Jimenez 1990). We have reported (Zicha and Kuneš 1994) that BP of DOC-treated rats fed $NaHCO_3$-containing diet can reach relatively high values, although the development of hypertension was slower compared to animals fed equimolar NaCl diet.

The different hypertensive effects of NaCl and $NaHCO_3$ in DOC-treated animals could not be attributed to the differences in either sodium and potassium balance (Kurtz and Morris 1983, Passmore *et al*. 1985) or total carcass sodium and potassium content (Passmore *et al*. 1985). NaCl-loaded, DOC-treated rats with elevated blood pressure were characterized by expanded extracellular fluid volume as compared to DOC-treated rats fed a high sodium, low-chloride diet (Passmore *et al*. 1985). In addition, DOC-induced hypokalemia was less pronounced in NaCl-loaded rats than in animals subjected to selective sodium loading (Kurtz and Morris 1983, Passmore *et al*. 1985).

The aim of our study was to determine the influence of loading with $NaHCO_3$ and NaCl, which were administered either in the diet or in the drinking fluid, on the development of DOC hypertension in young rats that are known to be highly susceptible to various forms of NaCl-dependent experimental hypertension (Zicha *et al.* 1986, Zicha and Kuneš 1999). The size of intravascular and interstitial compartments of extracellular fluid was determined in all experimental groups in order to elucidate the possible role of body fluid alterations in different BP response of DOC-treated rats subjected to various forms of selective sodium loading.

Methods

Seventy-four young male Wistar rats were uninephrectomized at the age of 28 days and treated with deoxycorticosterone acetate (40 mg kg^{-1} b.w., i.m., twice a week). Animals were randomly divided into five experimental groups according to the dietary regimen. Control rats of the first group were drinking tap water and were fed a low-salt, natural ingredient diet containing 25 mmol Na^+, 98 mmol K^+ and 47 mmol Cl^- kg^{-1} diet. Animals in the groups 2 and 3 were also fed this low-salt diet but were drinking *ad libitum* $NaHCO_3$ and NaCl solutions (170 mmol l^{-1}) instead of water. Rats of the groups 4 and 5 were kept on tap water but they were fed *ad libitum* diets supplemented with $NaHCO_3$ or NaCl in equimolar amounts (170 mmol Na^+ kg^{-1} diet). Fluid intake and diet consumption were measured throughout the experiment.

After 5 weeks of the experiment blood pressure was measured by the direct puncture of carotid artery under light ether anesthesia using P23Db pressure transducer (Statham, Hato Rey, PR, USA) and recorder HP7754A (Hewlett Packard, Andover, MA, USA). Subsequently, body fluid compartments were estimated as previously described in details (Kuneš 1989, Zicha *et al.* 1989). Briefly, plasma volume (PV) was determined by the dilution of Evans blue (1 ml of 0.5 % solution per kg b.w., Fluka, Buchs, Switzerland) and extracellular fluid volume (ECFV) was measured as the distribution space of polyfructosane (0.2 ml of 25 % solution per kg b.w., Inutest, Laevosan, Linz, Austria). Both indicators were administered into the jugular vein. Interstitial fluid volume was calculated by subtracting PV from ECFV values and blood volume was calculated from PV and hematocrit values. Plasma sodium and potassium concentrations were determined by means of Varian atomic absorption spectrophotometer and plasma osmolality using Knauer semimicroosmometer.

Data were expressed as means ± S.E.M. and evaluated by one-way-analysis of variance with the calculation of least significant differences (Snedecor and Cochran 1968). $P<0.05$ value was considered as significant.

Results

Dietary loading of rats with either NaCl or $NaHCO_3$ did not alter body weight (245±5 and 236±2 g, respectively) compared to controls (242±4 g). On the other hand, drinking of 1 % NaCl or 1.44 % $NaHCO_3$ solutions reduced substantially the body weight (174±5 and 170±11 g, respectively).

Though sodium intake was about six times higher in rats that were loaded with NaCl in the drinking fluid than those consuming NaCl diet, mean arterial pressure (MAP) was increased to a similar extent in both DOC-treated NaCl-loaded groups (Fig. 1). This was in contrast with the effects of $NaHCO_3$ which caused a considerable MAP increase only when it was administered in the diet.

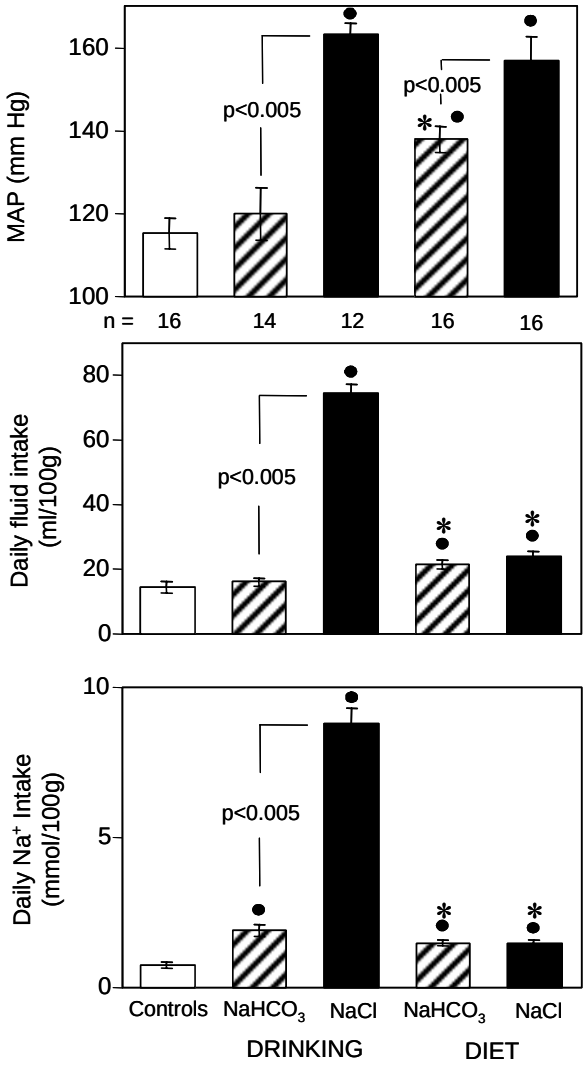

Fig. 1. Mean arterial pressure (MAP) as well as average fluid and sodium intake in young DOC-treated rats subjected to either selective sodium loading ($NaHCO_3$, cross-hatched bars) or concomitant sodium and chloride loading (NaCl, solid bars) for 5 weeks. Number of animals is indicated at the bottom of each column. Data are means ± S.E.M. Full dots represent significant differences ($p<0.005$) of the respective experimental group in comparison with the control group (open bars). Asterisks indicate the differences between animals loaded with the respective sodium salt in the diet and in the drinking fluid.

The absence of a significant MAP response in rats that were drinking of $NaHCO_3$ solution could be related to their insufficient fluid consumption. It should be noted that sodium intake in rats drinking $NaHCO_3$ solution was greater than that of animals fed $NaHCO_3$ or NaCl diets but their intake of water available for organism hydration and solute excretion was not adequate (Fig. 1).

Multiple alterations of body fluids disclosed in particular groups are shown in Tables 1 and 2. Plasma sodium concentration and plasma osmolality were increased in rats drinking 1 % NaCl or 1.44 % $NaHCO_3$ solutions but not in rats subjected to dietary NaCl or $NaHCO_3$ loading. DOC-induced hypokalemia was pronounced in both $NaHCO_3$-loaded groups (Table 1). There was a significant expansion of extracellular fluid volume in all experimental groups compared to controls but the degree of extracellular fluid volume expansion was greater in NaCl-loaded than in $NaHCO_3$-treated animals (Table 2). The same was true for the interstitial fluid volume. Though plasma volume (PV) was also enlarged in all experimental groups (Table 2), the highest PV values were found in rats drinking 1 % NaCl solution in which hematocrit was substantially reduced (Table 1). Blood volume was increased in most experimental groups (Table 2) except of $NaHCO_3$ drinking rats which failed to respond by blood pressure increase to DOC treatment (Fig. 1).

Discussion

In general, our findings are not contradictory to numerous previous reports on the important role of chloride in the pathogenesis of salt-dependent hypertension (Kotchen *et al.* 1983, Whitescarver *et al.* 1984, Kurtz and Morris 1983, 1985, Whitescarver *et al.* 1986a, Passmore *et al.* 1985). We have confirmed that combined sodium and chloride loading was more efficient in the induction of DOC hypertension than selective sodium loading. Our present results obtained in highly susceptible young rats consuming $NaHCO_3$ diet are in a good agreement with earlier observations (Passmore *et al.* 1985, Motoyama *et al.* 1987, 1988) of a moderate blood pressure increase (by 20-30 mm Hg) induced by selective dietary sodium loading in DOC-treated rats. On the other hand, a non-significant MAP elevation by 5 mm Hg in our rats drinking $NaHCO_3$ solution is nearly identical with blood pressure change reported by Kurtz and Morris (1983) in their DOC-treated rats drinking $NaHCO_3$ solution.

Guyton *et al.* (1972) suggested that sodium retention, extracellular fluid volume enlargement and blood volume expansion play a key role in the pathogenesis of hypertension induced by high salt intake in individuals with reduced renal mass and/or mineralocorticoid administration. Some observations of increased blood volume and/or extracellular fluid volume in DOC-salt hypertensive rats (Jelínek 1972, Haack *et al.* 1977) support this concept. In our study blood volume was significantly increased in all groups of DOC-treated

rats subjected to high sodium intake with the exception of rats drinking $NaHCO_3$ solution in which no significant blood pressure increase occurred within 5 weeks of the experiment. In contrast, blood volume was expanded in rats consuming $NaHCO_3$ diet which developed moderate DOC hypertension. This was the main difference between both groups of animals subjected to different forms of selective sodium loading.

Table 1. Plasma sodium (P_{Na}) and potassium (P_K) concentrations, plasma osmolality (P_{osm}) and hematocrit (Htc) in DOC-treated rats loaded with NaCl or $NaHCO_3$ offered in the drinking fluid or in the diet

Group	n	P_{Na} mmol.l^{-1}	P_K mmol.l^{-1}	P_{osm} mosmol.l^{-1}	Htc %
Controls	16	144±2.1	3.50±0.19	318±1.5	52.6±0.84
1 % NaCl solution	12	159±4.8**	4.01±0.21	339±6.0*	40.0±1.00**
1.44 % $NaHCO_3$ solution	14	152±0.6*	2.40±0.15^{++}	343±5.0**	48.4±0.40*
1 % NaCl diet	16	139±2.4$^{\$\$}$	3.35±0.15$^{\$}$	317±2.1$^{\$\$}$	51.1±0.86$^{++\$\$}$
1.44 % $NaHCO_3$ diet	16	142±2.7$^{\$}$	2.56±0.23^{*+}	320±5.2$^{\$\$}$	50.6±1.41
$F_{4,69}$		8.56	11.72	8.89	22.69
$p<$		0.001	0.001	0.001	0.001
LSD (p=0.05)		7.5	0.53	11.6	2.77

Significantly different (p<0.05, p<0.001): *, ** vs controls; $^{+}$, $^{++}$ vs NaCl; $^{\$}$, $^{\$\$}$ diet vs respective drinking fluid; LSD - least significant difference at 5 % level

Table 2. Extracellular fluid volume (ECFV), plasma volume (PV), interstitial fluid volume (IFV) and blood volume (BV) in DOC-treated rats loaded either with NaCl or $NaHCO_3$ offered in the drinking fluid or in the diet

Group	n	ECFV	PV	IFV	BV
		ml.100g^{-1} body weight			
Controls	16	17.03±0.50	3.34±0.07	13.69±0.53	5.90±0.11
1 % NaCl solution	12	23.09±0.60**	4.67±0.17**	18.33±0.60**	7.04±0.23**
1.44 % $NaHCO_3$ solution	14	19.77±0.22^{**++}	3.70±0.07^{*++}	16.06±0.22^{**++}	6.17±0.15^{++}
1 % NaCl diet	16	21.65±0.28 $^{**\$}$	3.69±0.09 $^{*\$\$}$	17.97±0.31**	6.38±0.16 $^{*\$}$
1.44 % $NaHCO_3$ diet	16	19.18±0.34^{**++}	3.79±0.10 *	15.38±0.33^{*++}	6.52±0.17*
$F_{4,69}$		32.9	21.6	21.6	4.10
$p<$		0.001	0.001	0.001	0.01
LSD (p=0.05)		1.13	0.28	1.16	0.4

Significantly different (p<0.05, p<0.001): *, ** vs controls; $^{+}$, $^{++}$ vs NaCl; $^{\$}$, $^{\$\$}$ diet vs respective drinking fluid; LSD - least significant difference at 5 % level

On the other hand, in both $NaHCO_3$-loaded groups there was a moderate increase of extracellular fluid volume which was, however, substantially smaller than that found in NaCl-loaded rats. This is in accordance with the findings of Passmore *et al.* (1985) who observed that the restriction of chloride intake prevented expansion of extracellular fluid volume in DOC-treated animals. DOC-induced hypokalemia was reported to be augmented by alkalosis which accompanied selective sodium loading without chloride (Kurtz and Morris 1983, Passmore *et al.* 1985). This was also true in our DOC-treated rats with $NaHCO_3$, but the degree of hypokalemia was similar in both groups irrespective of their blood pressure response.

Thus among all body fluid alterations disclosed in our study the changes of intravascular volume seem to be most relevant to the observed blood pressure changes. These results represent the direct experimental support

for a hypothesis of Boegehold and Kotchen (1989) about the importance of intravascular expansion for the hypertensive effects of sodium chloride as compared to non-chloride sodium salts. This hypothesis was based upon the data obtained in salt-sensitive humans in which plasma volume was greater on a high NaCl diet than on a sodium citrate diet (Kurtz *et al.* 1987).

It should, however, be noted that the failure of DOC-treated rats drinking $NaHCO_3$ solution to increase blood pressure might be ascribed not only to the absence of intravascular expansion but also to a certain degree of dehydration resulting from a low fluid consumption in this experimental group. Our earlier studies (Zicha *et al.* 1989, Kuneš *et al.* 1989) as well as those by Hofbauer *et al.* (1984a,b) indicated that sufficient water retention is a necessary prerequisite for the development of salt-dependent forms of experimental hypertension in the rat because isolated sodium retention did not permit the development of DOC-salt hypertension in dehydrated animals (Kuneš *et al.* 1989).

On the other hand, smaller BP elevation in DOC-treated rats consuming $NaHCO_3$ diet than in those fed NaCl diet cannot be solely ascribed to the different degree of body fluid expansion. We have demonstrated that DOC-treated rats subjected to dietary selective sodium loading are characterized by the absence of changes in arterial compliance which prevents the augmentation of pulse pressure seen in DOC-NaCl hypertensive rats (Zicha and Kuneš 1994). Moreover, the activation of sympathetic nervous system seems to be attenuated in DOC-treated rats fed $NaHCO_3$ diet compared to those consuming NaCl diet (Govyrin *et al.* 1986). This would explain our observation (Zicha and Kuneš 1994) of a slower rise in systemic resistance found in DOC-treated rats subjected to dietary selective sodium loading compared to NaCl-loaded animals.

Our findings demonstrated that selective sodium loading under the appropriate conditions ($NaHCO_3$ diet) could elicit a moderate blood pressure elevation in young DOC-treated rats.

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