Separate Hemodynamic Roles for Chloride and Sodium in Deoxycorticosterone Acetate-Salt Hypertension (43092)

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Abstract. **It has been reported that both sodium and chloride ions must be injested to induce the elevated blood pressure of deoxycorticosterone acetate (DOCA)-salt-sensitive hypertension. This study was designed to determine the separate roles of the sodium and chloride ions in the altered hemodynamics underlying the high blood pressure. DOCA pellets (75 mg) were implanted in uninephrectomized rats and the animals were then fed one of four diets: (i) high sodium chloride, (ii) high sodium-low chloride, (iii) high chloride-low sodium, or (iv) low sodium chloride. Blood pressures were measured weekly by tail-cuff plethysmography for 5 weeks and the animals were then subjected to a terminal experiment to measure cardiac output by thermodilution technique, renal blood flow by electromagnetic flow probe, and direct arterial pressure. Blood pressure in the DOCA-high NaCl group was significantly greater ($P<0.05$) compared with that of the DOCA-low NaCl group (160 ± 3 mm Hg vs 124 ± 2 mm Hg, respectively) at 5 weeks after treatment; all other groups were not significantly different from the DOCA-low NaCl group. Cardiac output was significantly greater in DOCA-treated rats consuming diets high in sodium (44 ± 2 ml/min/100 g) or sodium chloride (40 ± 2 ml/min/100 g) compared with animals consuming low sodium chloride (31 ± 2 ml/min/100 g; $P < 0.01$ for each comparison). Direct intraarterial blood pressure and renal blood flow were used to calculate renal vascular resistance. Renal vascular resistance was increased in those DOCA-treated rats consuming diets high in chloride (42 ± 3 mm Hg/ml/min/100 g) and high sodium chloride (54 ± 3 mm Hg/ml/min/100 g) compared with rats consuming low sodium chloride (30 ± 3 mm Hg/ml/min/100 g; $P < 0.01$ for each). It appears that elevations in cardiac output are associated with increased dietary sodium and act in synergy with the elevations in renal vascular resistance associated with increased dietary chloride. Increases in both cardiac output and renal vascular resistance are involved in the maintenance of elevated blood pressure in the DOCA-salt-sensitive model of hypertension.** [P.S.E.B.M. 1990, Vol 194]

The mechanisms by which high dietary sodium chloride induces hypertension are not completely known. High dietary sodium alone (provided as nonchloride-containing compounds) does not induce a blood pressure rise in salt-sensitive hypertensive patients (1) nor in the genetic (Dahl-S) and deoxycorticosterone acetate-salt-sensitive (DOCA-salt) rat models of hypertension (2–5). Sodium and chloride must both be present to induce the blood pressure rise in "salt-sensitive" hypertension and therefore it is possible that they have separate and unique roles in the hypertensive process.

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We have shown that the presence of high chloride in the diets of DOCA-implanted rats is associated with a reduced renal blood flow. These results are consistent with the findings of Wilcox (6) in which chloride infused directly into the renal artery of anesthetized dogs induced a reduction in renal blood flow and glomerular filtration rate. It is our hypothesis that chloride at the renal vasculature elevates renal vascular resistance and provides a significant portion of the increased total peripheral resistance of DOCA-NaCl-induced hypertension.

Cardiac output and total peripheral resistance are the factors that determine mean arterial blood pressure and are related by the formula: MAP = CO × TPR. This relationship suggests that a rise in mean arterial blood pressure will occur when either cardiac output or total peripheral resistance are increased, unless there is

a decrease in the other parameter (7). This study investigates the relationship between cardiac output and renal vascular resistance in causing DOCA-salt hypertension in rats. We have determined hemodynamic events which characterize the separate and unique roles of sodium and chloride in causing blood pressure elevation in this form of hypertension.

Materials and Methods

Male Sprague-Dawley rats (75–100 g) from Charles River were housed one per cage in light (14L:10D)- and temperature (23°C)-controlled rooms. Rats were anesthetized with methoxyflurane and uninephrectomized. At 12 days after surgery, animals in DOCA-treated groups then received a 75-mg DOCA pellet subcutaneously while under light anesthesia and on the same day were started on one of the four special salt diets described below. The DOCA pellets were replaced every 2.5 weeks.

Tail-cuff blood pressures were obtained prior to DOCA implant and twice a week for the 40 to 45 days of DOCA/diet treatment. The animals were warmed on a heating pad and the average of three tracings was determined. The precautions outlined by Bunag (8) were observed during these measurements. We have previously published evidence that we find a reliable correlation between tail-cuff measurements and the direct intraarterial blood pressure measurements obtained during the terminal experiments (3).

Preparation of Diets. To formulate the desired level of sodium in the diet, sodium as sodium chloride or sodium with assorted anions other than chloride was added (3, 4) to a special low sodium, low chloride diet which was obtained from Ralston Purina Co. (Table I). Chloride as sodium chloride or chloride with assorted cations other than sodium (5) was added to the basic Purina low NaCl diet to provide desired chloride contents. Potassium was added in the form of various potassium compounds to provide a total of 0.61 mEq of K^+/g of food (Table I). We have previously determined that plasma potassium levels in DOCA-salt-treated rats are maintained near normal with this amount of potassium supplementation (3).

The DOCA-high NaCl group received DOCA pellets and 1.11 mEq of sodium/g of food with 0.75 mEq of chloride/g in the diet for 40 to 45 days. The DOCA-high Na^+ group received DOCA and 1.15 mEq of sodium/g of food with 0.05 mEq of chloride/g of food in the diet for 40 to 45 days. The DOCA-high Cl^- group received DOCA and 0.09 mEq of sodium/g of food with 0.75 mEq of chloride/g of food in the diet for 40 to 45 days. The DOCA-low NaCl received DOCA and 0.09 mEq of sodium/g of food with 0.05 mEq of chloride/g of food in the diet for 40 to 45 days.

We have previously shown that 1.1 mEq/g of sodium in the high sodium diets to be an appropriate level of sodium chloride for the induction of DOCA-salt hypertension (3). It has been previously demonstrated (5) that rats will consume a diet containing 0.75 mEq/g of chloride; we chose to use that as our high chloride diet. To compare the high sodium chloride fed animals with the high sodium animals or the high chloride animals, we used equivalent amounts of sodium and chloride in the high sodium chloride diet, i.e., 1.1 mEq/g of sodium and 0.75 mEq/g of chloride.

After the animals in each group had been receiving DOCA and diet for 40 to 45 days, each animal was anesthetized with pentobarbital (45 mg/kg i.p.) and subjected to a terminal experiment to measure one or more of the following parameters.

Measurement of Cardiac Output and Arterial Blood Pressure. The rat was anesthetized with pento-

Table I. Ionic Contents of Diets for DOCA-Treated Rats (mEq/g Rat Chow)[a]

Group	Added	Basic diet	Total
High NaCl (5.2%)			
NaCl	0.74		
Sodium Glycinate	0.36		
Na^+		0.01	1.11
Cl^-		0.01	0.75
$KHCO_3$	0.17		
K_2SO_4	0.17		
KH_2PO_4	0.17		
K^+		0.1	0.61
High Na			
NaCl	0.04		
NaH_2PO_4	0.05		
Sodium aspartate (mono)	0.21		
$NaHCO_3$	0.15		
Sodium glutamate (mono)	0.22		
Sodium glycinate (mono)	0.47		
Na^+		0.01	1.15
Cl^-		0.01	0.05
$KHCO_3$	0.17		
K_2SO_4	0.17		
KH_2PO_4	0.17		
K^+		0.1	0.61
High Cl^-			
NaCl	0.08		
KCl	0.51		
Glycine HCl	0.15		
Na^+		0.01	0.09
Cl^-		0.01	0.75
K^+		0.1	0.61
Low NaCl (0.4%)			
NaCl	0.04		
Sodium glycinate (mono)	0.04		
Na^+		0.01	0.09
Cl^-		0.01	0.05
$KHCO_3$	0.17		
K_2SO_4	0.17		
KH_2PO_4	0.17		
K^+		0.1	0.61

[a] All diets were prepared from a low sodium/low chloride purified diet obtained from Purina Mills Inc. (St. Louis, MO; 5882M-5) which contains 0.01 mEq of NaCl/g rat chow, 0.10 mEq of K^+/g, 0.15 mEq of Ca^{2+}/g, and 0.13 mEq of phosphorous/g.

barbital and placed in a supine position on a thermostatically controlled heating mat to maintain rectal temperature between 37°C and 38°C. The trachea was cannulated with a short segment of PE-240 tubing. The right jugular vein was cannulated with PE-60 tubing inserted to the level of the right atrium. A thermister (Yellow Springs Instrument Co., Inc.) was positioned in the right common carotid artery at the aortic root. Cardiac output was measured from thermal dilution curves generated by the bolus injection of 80 μl of room temperature saline via the right jugular into the right atrium. Cardiac output was calculated by using the appropriate formulas (9, 10) and the area under the thermal dilution curve. After determination of cardiac output, the termister was removed. An arterial cannula was inserted and arterial blood pressure was measured via the carotid artery (Strain gauge, Medex model 840); Medex Inc., Hilliard, Ohio) at the end of the experiment.

Measurement of Renal Blood Flow and Arterial Blood Pressure. A left flank incision was made for the placement of an electromagnetic flow probe (Carolina Medical Electronics, King, North Carolina) on the renal artery to measure total renal blood flow.

Measurement of Blood pH. A blood sample was taken from the carotid arterial cannula for measurement of blood pH after all other procedures were completed. The DOCA-salt model of hypertension is known to induce alkalosis (11) and a severe alkalosis could alter hemodynamic parameters. Although potassium supplementation partially prevents the alkalosis, we feel it important to measure and report pH to assure that pH alterations are not the basis for changes in cardiac output and renal blood flow between groups.

Statistics. Two-way analysis of variance was used to determine significant differences in blood pressure among groups over time. One-way analysis of variance was used to determine significant differences in body weight, cardiac output, direct arterial pressure, renal blood flow, and renal vascular resistance among groups. For multiple comparisons among groups, the Bonferroni method of modified t statistics was applied following analysis of variance (12).

Results

All animals gained weight and appeared to be healthy at the time of sacrifice (Table II). DOCA-high NaCl rats gained 108 ± 9 g during the time of DOCA and diet administration and weighed 362 ± 8 g at the time of sacrifice. DOCA-high Na^+ rats gained significantly less weight than did the DOCA-low NaCl animals ($P < 0.01$) and weighed significantly less at 315 ± 7 g at the time of sacrifice ($P < 0.05$). Rats in the DOCA-high Cl^- and DOCA-low NaCl groups gained 101 ± 13 and 131 ± 21 g, respectively, and weighed 357 ± 11 and 380 ± 14 g, respectively, at sacrifice.

Table II. Weights (g) of DOCA-Treated Rats Consuming Diets with Various Sodium and Chloride Contents

	Weight at sacrifice	Weight gain during experiment
DOCA-high NaCl (12)[a]	362 ± 8	108 ± 9
DOCA-high Na^+ (12)	315 ± 7[b]	67 ± 10[c]
DOCA-high Cl^- (9)	357 ± 11	101 ± 13
DOCA-low NaCl (5)	380 ± 14	131 ± 21

[a] Numbers in parentheses, number in group.
[b] $P < 0.05$ significantly different from the DOCA-low NaCl group.
[c] $P < 0.01$ significantly different from the DOCA-low NaCl group.

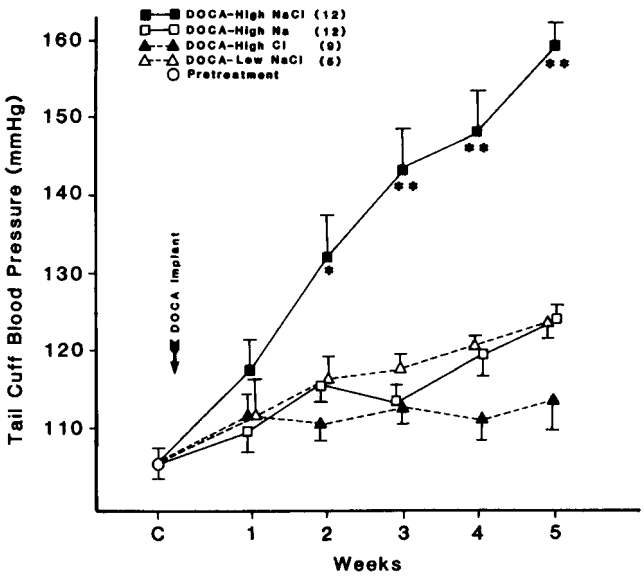

Figure 1. Tail-cuff blood pressures taken weekly on the four groups of DOCA-diet-treated rats. Values are mean ± SE. * $P<0.05$ compared with DOCA-low NaCl group; ** $P<0.01$ compared with the DOCA-low NaCl group. Numbers in parentheses, number in each group.

DOCA-high NaCl rats demonstrated a significant and greater elevation of blood pressure compared with that of the DOCA-low NaCl group as determined by the tail-cuff method by 2 weeks of treatment. The increased pressure was sustained throughout the 5-week experimental period as indicated by two-way analysis of variance ($p < 0.025$; Fig. 1). Neither the DOCA-high Na^+ group nor the DOCA-high Cl^- group had a significant elevation of tail-cuff blood pressure above that of the DOCA-low NaCl group. The blood pressures of the DOCA-high Na^+ and high Cl^- groups were significantly lower than those of the DOCA-high NaCl groups at 3–5 weeks ($P < 0.01$, not indicated in Fig. 1). Direct arterial pressure was significantly increased in the DOCA-high NaCl and DOCA-high Na^+ groups compared with that of the DOCA-low NaCl group ($P < 0.01$, Table III). Direct blood pressure of the DOCA-high Na^+ group was midway between those of the DOCA-high NaCl and DOCA-low NaCl groups.

Table III. Direct Arterial Blood Pressure and Renal Blood Flow for DOCA-Treated Rats Consuming Diets with Various Sodium and Chloride Contents.

Group	Direct blood pressure (mm Hg)	Renal blood flow (ml/min/100 g)
DOCA-high NaCl	151 ± 4[a] (9)[b]	2.9 ± .1 (10)
DOCA-high Na^+	129 ± 4[a,c] (9)	4.3 ± .3[c] (8)
DOCA-high Cl^-	106 ± 3 (10)	2.5 ± .1[a,c] (7)
DOCA-low NaCl	109 ± 4 (10)	3.7 ± .2 (5)

[a] $P < 0.01$ significantly different from the DOCA low NaCl group.
[b] Numbers in parentheses, number in group.
[c] $P < 0.05$ significantly different from the DOCA-high NaCl group.

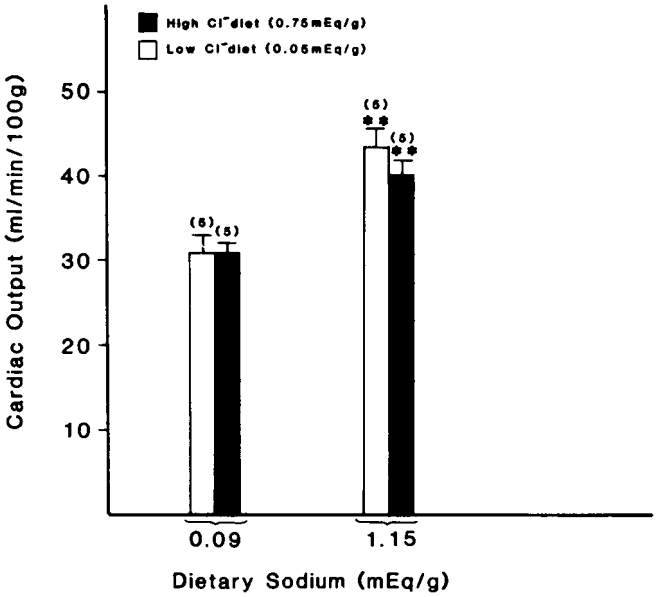

Figure 2. Cardiac output determined by the thermodilution technique for the four dietary groups of DOCA-treated rats. **$P<0.01$ compared with the DOCA-low NaCl group. Numbers in parentheses, number in each group.

The DOCA-high NaCl rats and the DOCA-high Na^+ rats had significantly elevated cardiac outputs compared with those of the DOCA-low NaCl ($P < 0.01$ for each; Fig. 2). Cardiac output for the DOCA-high Cl^- group was not significantly different from that of the DOCA-low NaCl group. The DOCA-high Na^+ rats had cardiac outputs which were equivalent to those of the DOCA-high NaCl animals.

The DOCA-high NaCl group of rats demonstrated significantly reduced renal blood flow compared with that of the DOCA-low NaCl group of rats ($P < 0.01$, Table III). The DOCA-high Cl^- rats had significantly lower renal blood flows than those of the DOCA-low NaCl ($P < 0.01$) and the DOCA-high NaCl groups ($P < 0.05$). The DOCA-high Na^+ groups of rats had renal blood flow values that were not significantly different from those of the DOCA-low NaCl group but were significantly greater than those of the DOCA-high NaCl group ($P < 0.05$).

Renal vascular resistance was significantly greater in the DOCA-high NaCl and in the DOCA-high Cl^- group than in the DOCA-low NaCl group ($P < 0.01$, Fig. 3). Renal vascular resistance was significantly lower in the DOCA-high Cl^- group compared with the DOCA-high NaCl group ($P<0.05$). Renal vascular resistance in the DOCA-high Na^+ group was not significantly different from that of the DOCA-low NaCl group. At the time of sacrifice, DOCA-high NaCl, DOCA-high Na^+, DOCA-high Cl^-, and DOCA-low NaCl rats had blood pH values of 7.52 ± 0.03, 7.58 ± 0.02, 7.45 ± 0.15, and 7.47 ± 0.02, respectively.

Discussion

The increase in blood pressure of hypertensive individuals ingesting sodium chloride has led to the concept that certain forms of hypertension are salt dependent. Sodium was considered to be the primary effective ion in the induction of hypertensive pathophysiology (13); however, more recent studies have demonstrated that sodium must be provided as sodium chloride to induce salt-sensitive hypertension (1–4). In addition, it has been shown that elevated dietary chloride without elevated sodium does not induce hypertension in Dahl-S rats (5). Our study now reports that elevated dietary chloride alone did not cause elevated blood pressure in the DOCA model of hypertension. The fact that elevation of either dietary sodium or chloride alone will not cause hypertension implies that the two ions need to be provided together and thus

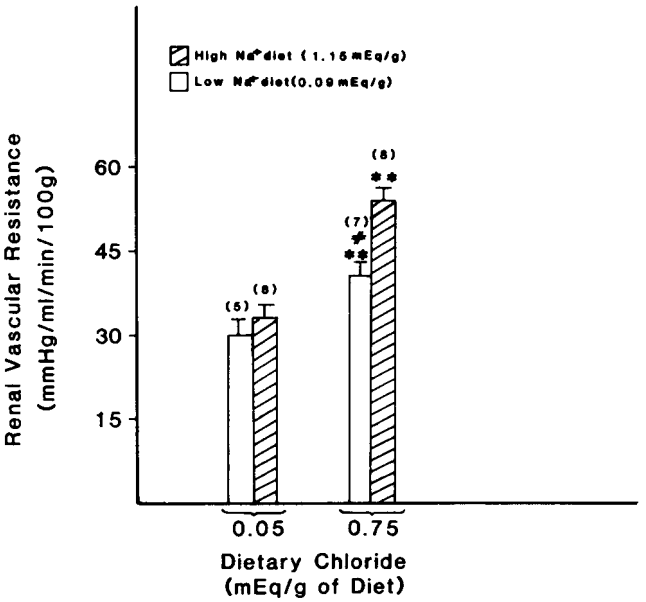

Figure 3. Renal vascular resistance calculated from direct arterial blood pressure and renal blood flow for the four dietary groups of DOCA-treated rats. **$P<0.01$ compared with the DOCA-low NaCl group; #$P<0.05$ compared with the DOCA-high NaCl group. Numbers in parentheses, number in each group.

have a (cooperative) synergistic effect in the augmentation of hypertension.

With the addition of various anions and cations to the diet, one must consider the possibility that these ions may affect the measured hemodynamic parameters. Dietary sodium concentration was equivalent in the high sodium chloride and high sodium diets and it also was equal in the low sodium chloride and high chloride diet (Table I). Chloride concentration was equivalent in the high sodium chloride and high chloride diets, and was at a same low level in the low sodium chloride and high sodium diets. Potassium concentration was equivalent in all four diets. It has been reported that potassium does have a protective effect on the development of hypertension (14) as well as protection against alkalosis and hypokalemia (11). The assorted anions associated with the potassium supplementation were constant for three diets (high NaCl, high Na, and low NaCl), and it is unlikely that any of these were the ions responsible for observed differences between groups. Amino acids and the minimal concentrations of any other electrolytes were also unlikely causative factors and were distributed across more than one diet. It has been demonstrated that our manipulation of the dietary electrolytes has no depressive effect on blood pressure in the one kidney:one clip model of renal hypertension (3–5). Also, we have reported similar effects of selective dietary loading on blood pressure and renal blood flow in the angiotensin II model of salt-sensitive hypertension (15). We propose that the observed hemodynamic differences in this study are due primarily to manipulation of dietary sodium and chloride.

It is our experience that DOCA-treated animals gain weight at a slightly slower rate than untreated rats; however, the DOCA-treated animals were within the normal weight range for age. In a previous study (3), the weight of the animals in the DOCA-high Na^+ group was not significantly less than that of DOCA-high NaCl group, thus we believe that the difference in weight in the present study is not a detrimental factor. All values for cardiac output and renal blood flow were expressed per 100 g body weight to account for differences in body weight.

Elevation of blood pressure can occur as a result of either an increased cardiac output and/or an increased peripheral resistance. Cohen *et al.* (16) reported that the cardiac output of pigs was increased 152% by the DOCA-high NaCl treatment. Previous studies demonstrate that chloride ions infused directly into the renal artery (6) or an increased renal tubular chloride load by microperfusion (17) can alter renal vascular resistance.

The results of the present study reveal that DOCA-treated rats consuming a diet of high sodium concentration had a greater cardiac output than rats consuming a diet containing low (normal) concentrations of sodium. This increase was associated with the increased sodium concentration whether the dietary chloride was elevated or not. The increased cardiac output in the DOCA-high Na^+ group was not associated with an increase in tail-cuff blood pressure. The directly measured mean arterial blood pressure of the DOCA-high Na^+ group rats was midway between those of the DOCA-high NaCl and DOCA-low NaCl. This finding suggests that the elevated dietary sodium alone partially elevates the systemic blood pressure of DOCA-treated rats. The mechanism may involve an effect of sodium to increase sympathetic drive. As we have previously reported (3) that the extracellular fluid volume is not expanded in the DOCA-high Na^+ rats, it seems likely that the increased cardiac output may be related to other actions of sodium. One site of action of sodium to increase cardiac output could be a central effect of an increase in sympathetic nervous activity (18). The effect of sodium chloride on blood pressure is prevented by hypothalamic lesions which disrupt descending sympathetic pathways (18). Regardless of the site of action, our results reveal a relationship between high dietary sodium and increased cardiac output in the DOCA model with or without an elevated blood pressure.

The results of our study also reveal that the consumption of increased amounts of dietary chloride by DOCA-treated rats is associated with increased renal vascular resistance. Previously, we reported that the DOCA-high Na^+ group of animals had reduced renal vascular resistance compared with DOCA-high NaCl (3); in this study we have shown that renal vascular resistance is increased in DOCA-high NaCl and also elevated in the DOCA-high Cl^- group in which dietary sodium is low. Therefore, the elevation in resistance appears to be related to the chloride intake. Since the DOCA-high Cl^- rats were normotensive, it appears that much of the increase in renal vascular resistance in this group may not be related to an autoregulatory effect of elevated blood pressure. However, the DOCA-high NaCl group had significantly higher renal vascular resistance than the DOCA-high Cl^- group, suggesting that an autoregulation mechanism may be associated with the elevated blood pressure and add to the elevation of renal vascular resistance in this group. It has been demonstrated that direct infusions of solutions containing chloride into the renal arteries of anesthetized dogs resulted in an immediate increase in renal vascular resistance (6) and, furthermore, chloride has been implicated in alterations in vascular resistance induced by tubuloglomerular feedback (17). Regardless of the mechanism, our results reveal a relationship between high dietary chloride and increased renal vascular resistance in DOCA-salt hypertension.

We have found that sodium as well as chloride must be provided in the diet for the blood pressure rise

in DOCA-salt hypertension. Sodium alone may partially elevate blood pressure but both ions must be present for maximum blood pressure rise. Elevated dietary sodium is associated with increased cardiac output while elevated dietary chloride is associated with increased renal vascular resistance in this model of salt-sensitive hypertension. These two hemodynamic factors representing separate and unique effects of sodium and chloride are most likely responsible for the maintenance of the blood pressure elevation following 6 weeks of DOCA-high NaCl treatment.

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