

# Effects of salt loading on sympathetic activity and blood pressure in anesthetized two-kidney, one clip hypertensive rats

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## ABSTRACT

In this study, we investigated the effects of salt loading on sympathetic pressor activity, cardiac autonomic activity, mean arterial pressure (MAP), heart rate (HR) and on the relations between them in anesthetized two-kidney, one clip (2K1C) hypertensive rats. We submitted rats to either renal artery clipping or sham operation. Distilled water or 0.5 % NaCl was given orally to the clipped and sham-operated control rats for 4 weeks. Then, MAP and HR differences between pre- and post- autonomic blockade were evaluated as indexes of sympathetic pressor and cardiac autonomic activity, respectively. The autonomic blockade decreased MAP to the similar levels in all groups (between  $81.7 \pm 7.6$  -  $87.3 \pm 7.1$  mmHg). Sympathetic pressor activity was greater in the clipped rats than in its sham-operated controls only under salt loading ( $55.3 \pm 6.2$  vs.  $37.0 \pm 4.1$  mmHg,  $p < 0.05$ ). Cardiac autonomic activity was, predominantly, sympathetic and more in the clipped group than in the sham-operated rats under distilled water ( $48.3 \pm 8.6$  vs.  $19.7 \pm 7.0$  beats/min,  $p < 0.05$ ) but not under salt loading. Salt loading inverted the relationship between HR and cardiac autonomic activity in 2K1C hypertensive rats ( $r = -0.76$ ,  $p = 0.046$  vs.  $r = 0.89$ ,  $p = 0.019$ ). These results suggest that salt loading may have augmented the effect of renovascular constriction on MAP by affecting the sympathetic pressor activity and the relation between cardiac autonomic activity and HR in 2K1C hypertensive rats.

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KEY WORDS: renovascular hypertension, sodium, angiotensin II, sympathetic nervous system

## INTRODUCTION

Two-kidney, one clip hypertension (2K1C) is primarily caused by high levels of angiotensin II (ANG II) [1, 2] and sympathetic activity [3]. Salt intake has the effects on sympathetic activity [4] beside renin angiotensin system [5]. For instance, a high salt diet increases the transmitter release in adrenergic neurons [4] as well as suppresses plasma renin activity in 2K1C hypertensive rats [6]. Salt intake condition may also affect sympathetic activity as a result of an interaction between ANG II and baroreflex [7]. When salt intake is low, high ANG II may reset the arterial baroreflex to higher operating pressures. This resetting is likely an important factor in the changes of sympathetic outflow [8]. The effect of salt on baroreflex is also significant, because baroreflex protects against increase in blood pressure (BP) after ANG II injection [9] and plays a significant role in the long-term regulation of BP under variable salt intake conditions [10]. In addition, according to two recent studies, high salt intake disrupts the normal sympathoinhibitory response to angiotensin II-based hypertension [11] and enhances the

responsiveness of rostral ventrolateral medulla neurons via ventral lamina terminalis [12]. Thus, due to the interaction between ANG II and baroreflex, a high salt diet can affect sympathetic activity both directly and indirectly. Since high salt intake has direct and indirect effects on sympathetic activity, as mentioned above, salt intake conditions may affect the development of 2K1C hypertension. However, the relationship between salt intake and sympathetic activity in this form of hypertension remains unclear. For this reason, we aimed to investigate the influence of salt loading on sympathetic pressor activity, cardiac autonomic activity, mean arterial blood pressure (MAP), heart rate (HR) and on the relations between them in anesthetized 2K1C hypertensive rats.

## MATERIALS AND METHODS

### *The animals and the clipping operation*

Experiments were carried out on the 30 normotensive male Wistar rats, weighing between 164 and 225 g, from the breeding unit of our university (Experimental Research Centre, Çukurova University, Adana, Turkey). The experimental protocols were approved by the animal care and use committee of the University of Çukurova. All experiments were performed according to the "Principles of Laboratory Animal Care" (NIH publication No. 85-23, revised 1985). The rats were housed four to a cage in stainless steel cages. All rats

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received standard rat chow (0.5% NaCl and 22% protein) ad libitum. The room temperature was maintained at a constant 22°C. A light was switched on from 8:00 a.m. to 8:00 p.m. In all the animals, the left renal artery was isolated after a mid-line abdominal incision under ketamine (130 mg/kg, im) and chlorpromazine (1.3 mg/kg) anesthesia. The rats were randomly assigned to one of two groups. In one group of rats (n=14), the left renal artery was constricted by a ring-shaped silver clip with 0.29 mm internal diameter. In the second (sham-operated) group (n=16), the artery was not constricted.

#### Diet treatment

Tap water was given to the animals for a one-week after the surgical operation. We then formed the following 4 groups according to the drinking water they received: 1) sham-operated rats + distilled drinking water (SD, n=7); 2) clipped rats + distilled drinking water (CD, n=6); 3) sham-operated rats + 0.5% NaCl drinking water (SS, n=9); 4) clipped rats + 0.5% NaCl drinking water (CS, n=8) groups. Salt loading was accomplished by giving 0.5% NaCl drinking solution to the rats. All the animals were fed with standard rat chow throughout the study. These different drinking water compositions were maintained for 4 weeks.

#### BP and HR measurements and autonomic blockade

Surgical trauma [13] and tethering [14] leads to sympathetic activation. Although all anesthetics also affects the neural condition of rats, the sympathetic activity in rats can be evaluated under sodium pentobarbital anesthesia [15, 16] because it does not affect tonic autonomic activity, in contrast its effect on autonomic reflex response [17]. We therefore preferred to measure tonic sympathetic pressor [18, 19] and cardiac autonomic [20] activities by means of autonomic blockade with hexamethonium under pentobarbital sodium anesthesia. A tracheostomy (PE-240 tubing, Clay Adams, Parsippany, NJ) and catheterizations (PE-50 tubing) of the left femoral and jugular veins as well as the left femoral artery were performed under sodium pentobarbital (Sigma, 50 mg /kg, i.p.) anesthesia 5 weeks after the clipping operation. The saline in the PE-50 tubings was heparinized (100 IU/ml). Insensible fluid losses were compensated with 0.9% NaCl via the jugular vein with an infusion pump (Cole Parmer Instrument, IL) at a rate of 20 µl/min during the experiment. A stabilization period was maintained for 30 minutes after the surgical procedure was completed. Throughout the experiment, BP was recorded from the femoral artery by means of a pressure transducer (Grass Model PT300) that was connected to an amplifier-oscillograph set (Model 7P122P, Grass Instrument CO, MA). The measurements at the end of the stabilization period were accepted as the baseline BP and HR values. One minute after obtaining these measure-

ments, hexamethonium bromide (25 mg/kg/0.5 ml saline) was injected into the animal as a bolus via the femoral vein. BP and HR were recorded 1, 2 and 3 minutes after the injection. In addition, BP signals were recorded onto a computer at 1-minute intervals by means of a data acquisition system (MP 100 System, BIOPAC Systems, Inc., Santa Barbara, CA). The information on autonomic activity level was obtained from the BP and HR differences between baseline and post-blockade values. The kidneys were isolated and weighed after sacrificing the animals by saturated KCl. We excluded from our study data for clipped rats whose left kidney weight was more than 95% or less than 25% of the right kidney weight.

#### Data analysis

Statistical analyses were conducted using SPSS program (SPSS Inc., Chicago, IL, USA). The differences between the groups were evaluated by two-way Anova test followed by independent t-test, when parametric test conditions were provided. The differences were analyzed by the Mann-Whitney U-test with adjusted  $\alpha$  levels under non-parametric test conditions. Wilcoxon matched pairs test with adjusted  $\alpha$  level for paired data, was used to analyze the BP and HR changes induced by hexamethonium bromide infusion and to assess other differences within groups. Spearman correlation analysis was applied to determine correlations between parameters. The results were expressed as means  $\pm$  SEM. All tests were performed as two-tailed tests and the values with  $p < 0.05$  were considered statistically significant.

## RESULTS

#### Basal BP and HR

There were no significant differences between the two clipped groups with regard to basal diastolic (DBP), mean and systolic (SBP) arterial blood pressures (Figure 1).

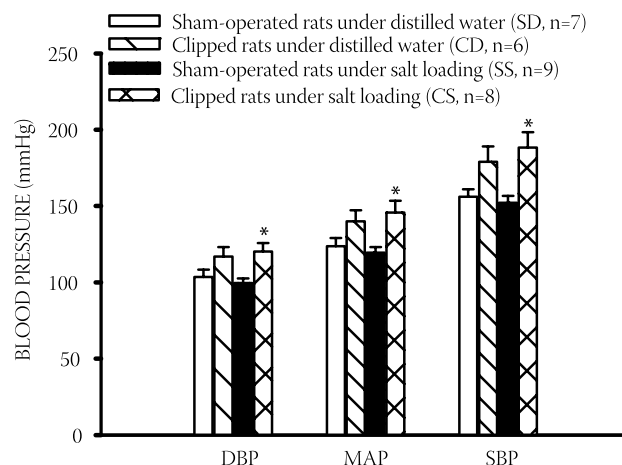
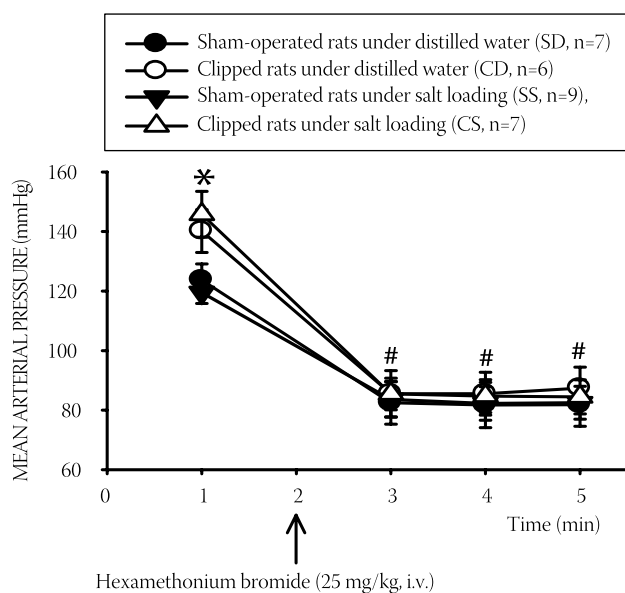


FIGURE 1. Baseline blood pressure in the clipped and sham-operated control rats. DBP: diastolic, MAP: mean and SBP: systolic blood pressures. Values are means  $\pm$  SEM. \*:  $p < 0.05$  vs. SS.



**FIGURE 2.** The effects of autonomic blockade on mean blood pressure (MAP). MAP values 1 min before and 1-3 min after autonomic blockade in the clipped and their sham-operated control rats. \*:  $p < 0.05$  for CS vs. SS.

DBP, MAP and SBP were higher in clipped rats than in sham-operated rats under salt loading ( $p < 0.05$ ) but only tended to be higher under distilled water intake. Basal HR was not significantly different among all the groups.

#### Effects of autonomic blockade on MAP

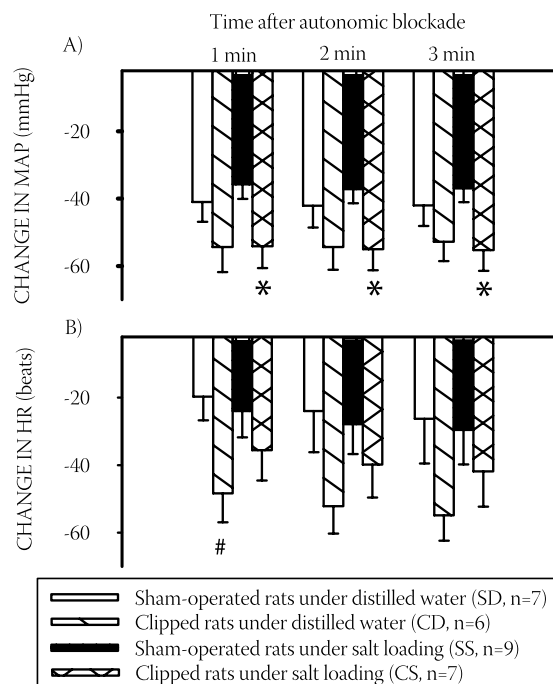
Autonomic blockade decreased MAP in all experimental groups ( $p < 0.05$ ) and led to disappear the significant MAP difference between clipped and sham-operated rats under salt loading (Figure 2). There was no significant difference between the two clipped groups with regard to the MAP decrease. MAP reduction was greater in the clipped group than in its sham-operated control under salt loading ( $p < 0.05$ ) but not under distilled water intake, for 3 minutes after the autonomic blockade (Figure 3A).

#### Effects of autonomic blockade on HR

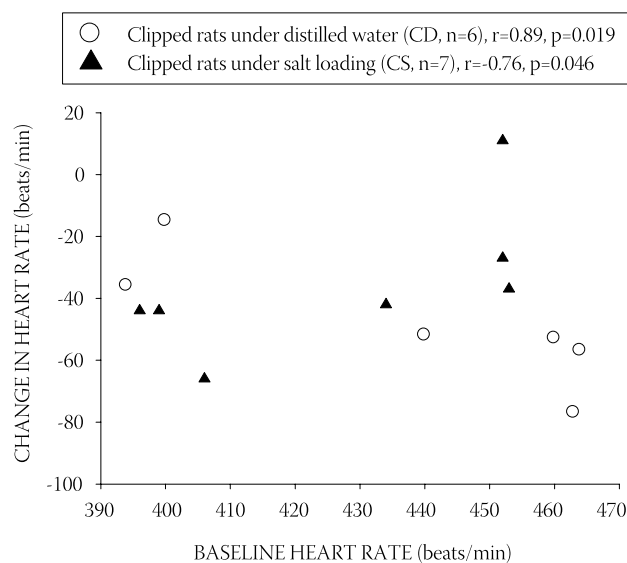
The decrease in HR was greater in the clipped group than in the sham-operated group at 1 minute after the blockade under distilled water ( $p < 0.05$ , Figure 3B) but this was not the case under salt loading. We did not find any other significant differences among the four experimental groups with regard to the HR change. Baseline heart rate was positively correlated with the change in heart rate 1 min after autonomic blockade in the clipped rats under distilled water ( $r = 0.89$ ,  $p = 0.019$ , Figure 4) and negatively correlated under salt loading ( $r = -0.76$ ,  $p = 0.046$ ). We did not find any significant correlation between the same parameters in the two sham-operated groups.

#### Renal weights

There were no significant differences between clipped rats



**FIGURE 3.** The amounts of change in blood pressure (MAP) and heart rate (HR) after the blockade. The amounts have been calculated for 1, 2 and 3 min after autonomic blockade in the clipped and their sham-operated control rats. \*:  $p < 0.05$  vs. SS; #:  $p < 0.05$  vs. SD



**FIGURE 4.** Correlation between baseline heart rate and the change in heart rate 1 min after the blockade in the clipped rat groups.

and its sham-operated controls with regard to left renal weights at the end of the 4 week diet-treatment period under both distilled water and salt loading conditions (Table 1). Right renal weight was greater in the clipped groups than in their respective sham-operated controls ( $p < 0.01$ ). Right renal weight was greater than left renal weight in both clipped groups ( $p < 0.01$ ). The two clipped groups did not significantly differ from each other with regard to right kidney weight.

**TABLE 1.** Kidney weights of study groups.

Parameter	Group			
	SD (n=7)	CD (n=6)	SS (n=9)	CS (n=8)
LRW (mg)	781±20.5	740±52.5	865±41.7	743±75.4
RRW (mg)	795±27.4	1054±58.6*†	858±39.7	1171±43.4#†

LRW (left renal weight) and RRW (right renal weight) are the values that were measured at the end of 4 weeks of different diets-treatment period. SD: sham-operated rats+distilled water, CD: clipped rats+distilled water, SS: sham-operated rats+0.5% NaCl solution, CS: clipped rats+0.5% NaCl solution groups. Values are means±SEM. \*  $p < 0.01$  v SD, #  $p < 0.001$  v SS and †:  $p < 0.05$  v LRW.

## DISCUSSION

Although previous studies investigated the effects of high salt intake on BP in 2K1C hypertensive rats [6] and on sympathetic activity in normotensive rats [4], our study is the first one that has elucidated the effect of salt loading on sympathetic pressor activity, cardiac autonomic activity and on its relationship with BP and HR in 2K1C hypertension. There are three important results of the present study. Firstly, salt loading condition led the clipping-induced increases in sympathetic pressor activity and MAP to be significant in anesthetized hypertensive rats. Secondly, the change in renin-angiotensin system was not sufficient to maintain hypertension in clipped rats without the contribution of an increase in peripheral sympathetic activity, under a salt loading condition. Thirdly, salt loading inverted the positive correlation between HR and cardiac autonomic activity in the clipped rats. The clipping reduced the kidney weight in a similar proportion under distilled water and salt loading. Thus, renovascular constriction caused an increase in MAP under salt loading. However, under distilled water intake there was only a tendency toward an increase in MAP. Given this difference, it is possible that salt loading may have augmented the effect of renovascular constriction on MAP. In fact, it has been reported that a high salt diet augmented [6] and a low salt diet decreased [21] BP in 2K1C hypertension. Nevertheless, there is also another report on renovascular constriction induced-hypertension under low salt intake [22]. The contrast among studies with regard to the effect of salt intake condition on BP in 2K1C hypertension may be due to the differences in BP measurement methods or clipping period. In the last study [22], BP had been measured by a tail cuff method which is less reliable than intra-arterial measurement [23]. In our other study, direct arterial blood pressure and plasma renin activity increased 3 weeks after unilateral renovascular constriction in conscious rats, without salt loading (unpublished data). Consequently, BP levels may change according to clipping period beside measurement method, in 2K1C hypertension. Although high ANG II activity in circulation has been suggested as the main factor leading to hypertension in the clipped rats

[24], plasma renin activity remains at a normal level 4 weeks after clipping in 2K1C hypertension [25]. This suggests that high local renin-angiotensin system activities (RAS) in the kidney [26] and the brain [27] and, enhanced slow pressor effect of angiotensin II [28] are possible factors in maintaining the hypertension. It has been suggested that ACE probably has an important role in development of renal mass compensatory mechanisms [29]. Studies have in fact shown that ANG II injected into cerebral ventricles attenuates cardiac baroreflexes [7]. The effect of local RAS on MAP may involve Ras/MAP kinase pathway because it has been reported that this pathway contributes to ANG-induced hypertension as well as ET-1 induced hypertension [30]. In addition, nitric oxide (NO) is an important mediator that opposes ANG II-induced hypertension [31]. NO is also a possible mediator for the hemodynamic changes associated with adaptations in renal mass [32]. For these reasons, changes in nitrergic control system [33, 34] may also contribute to this type hypertension. Moreover, salt loading may impair baroreflex [35] and sympathoinhibitory response to angiotensin II-induced hypertension [11]. Such impairments in pressure control mechanisms which are dependent on local ANG II activity or NO may contribute to maintaining high BP in clipped rats under salt loading. Blockade of the sympathetic nervous system with hexamethonium caused a significantly greater decrease in MAP in the clipped group than in sham-operated group under salt loading but not under distilled water. Thus, sympathetic hyperactivity was primarily responsible for maintaining hypertension under salt loading. This conclusion is consistent with other studies which found sympathetic hyperactivity in renovascular hypertension [36, 37]. ANG II enhances the release of norepinephrine in 2K1C hypertensive rats [38] and is a primary factor in maintaining BP level after sympathectomy in rats [39]. In our study, MAP decreased in the clipped and sham-operated groups after the blockade, causing the MAP values of the groups to approach each other. As a result, the significant difference between clipped and sham-operated rats under salt loading with regard to MAP disappeared after autonomic blockade. Therefore, the change in renin-angiotensin system can probably maintain hypertension in clipped rats with only the contribution of an increase in peripheral sympathetic activity under a salt loading condition. The central AT<sub>1</sub> receptor-mediated enhancement of cardiac sympathetic afferent reflex, contributes, in part, to the sympathetic activation and hypertension, according to a recent research by Zhu et. al. [40]. These conclusions are not in agreement with another study which suggests that 2K1C hypertension is maintained by the renin-angiotensin system without much contribution from the sympathetic nervous system [41]. Salt loading led the difference between clipped and sham-operated control rats with regard to sympathetic pressor activity to

be significant. Salt dependent augmentation in the clipping-induced sympathetic activation may be due to changes in the interaction between salt, RAS, the sympathetic system and baroreflexes. Baroreflex control is impaired in 2K1C hypertension [42]. When baroreflexes are impaired, an increase in salt intake may increase BP by affecting sympathetic activity via central neural mechanisms [43]. Moreover, high salt intake increases plasma norepinephrine levels [44], splanchnic sympathetic nerve activity in ANG II-induced hypertension [45] and lumbar sympathetic activity in salt-induced hypertension [46]. Because of these effects, salt loading may have caused the clipping-induced increase in MAP to be more prominent under salt loading than under distilled water, as a result of sympathetic potentiation. The HR decreases after the autonomic blockade was greater in the clipped group than in sham-operated group under distilled water but not under salt loading. Thus, it is possible that salt loading led to a decrease in the predominance of sympathetic drive to the heart in clipped rats. In addition, salt loading inverted the relationship between HR and cardiac autonomic activity in the clipped rats. In fact, salt dependent changes in adrenergic receptors [47] and baroreflex [35] may have caused to the changes in cardiac autonomic activity and its relationship with HR in clipped rats under salt loading. However, the salt did not significantly affect the basal HR level in the hypertensives. Consequently, the change in cardiac autonomic activity probably did not contribute to the salt induced augmentation of MAP in clipped rats. There was no significant difference between two clipped groups with regard to the difference between the left and right kidney weights at the end of the diet-treatment period. Consequently, salt loading did not influence the effect of clipping on renal mass. The effects of high salt on MAP and sympathetic activity may have developed without causing hypertrophy in the kidneys.

## CONCLUSION

In summary, we conclude that salt loading may have caused the clipping-induced increase in MAP to be more prominent as a result of the augmentation in sympathetic pressor activity. However, the change in cardiac autonomic activity probably did not contribute to the salt induced augmentation of MAP in clipped rats. Consequently, the change in renin angiotensin system can probably maintain hypertension in clipped rats only with the contribution of an increase in sympathetic pressor activity, under a salt loading condition.

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## DECLARATION OF INTEREST

There is no conflict of interest.

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