

CALCIUM SUPPLEMENT ENHANCES BAROREFLEX SENSITIVITY IN SALT-LOADED SPRAGUE-DAWLEY RATS

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SUMMARY: Dietary calcium is known to prevent salt-induced hypertension, although the exact mechanism responsible for this remains unknown. One of the proposed mechanisms of the pathogenesis of salt-induced hypertension is the impairment of baroreflex sensitivity. Hence we investigated the effect of calcium supplement on baroreceptor in salt-loaded rats. The experiment was performed in male Sprague-Dawley rats fed with measured salt and/or calcium diets and given tap water ad libitum for 6 weeks. Blood pressure and heart rate measurements were done in anaesthetised animals through direct invasive method using Grass Polygraph. Bilateral carotid occlusion test was used to determine the baroreflex sensitivity in the rats. There was increase in mean arterial pressure (MAP) of salt-loaded rats relative to control (132.6 ± 2.3 vs. 90.1 ± 1.5 mmHg; $n=8$; $p<0.05$), while dietary calcium alone did not have any significant effect on the MAP (84.5 ± 1.7 mmHg; $n=8$). During the feeding period, the salt consumed by salt-loaded rats was significantly higher than those of control but lower than that of salt-loaded-fed rats. Also, water intake was highest in salt-loaded rats compared with other experimental rats. However, the volume of urinary excretion was higher in salt-loaded-calcium-fed rats than salt-loaded rats but both were higher than control. These resulted in attenuated baroreflex sensitivity of salt-loaded rats relative to control (0.55 ± 0.2 vs. 1.25 ± 0.1 beats/min/mmHg, $n=7$; $P<0.05$). However, dietary calcium enhanced baroreflex sensitivity in salt-loaded-fed rats (2.21 ± 0.2 beats/min/mmHg, $n=7$; $P<0.05$) compared with control and salt-loaded rats. The study shows that salt-loading led to hypertension probably through alteration of haemodynamic function and impairment of baroreflex sensitivity. Calcium supplement prevention of salt-induced hypertension seems to reverse these, thus resulting in maintenance of water balance and baroreceptor integrity.

Key words: Salt-induced hypertension, baroreflex sensitivity, calcium supplement, Sprague-Dawley rats

Introduction

Although the precise mechanism which calcium prevents hypertension is yet to be established, it appears that salt-induced hypertension is the model of hypertension that is most sensitive to the effect of dietary calcium (Saito et al., 1989; Resnick, 1999). Experimental studies have shown that impairment of baroreceptor sensitivity in salt-loaded rats is responsible for the genesis of hypertension (Miyajima and Bunag, 1987; Sofola et al., 1991). There is evidence that abnormality in baroreceptor function precedes and contributes to elevation in arterial pressure (Mark, 1991). The resetting of the baroreceptor over a prolonged period is probably to enable it cope with the sustained elevation of the arterial pressure (Gordan et al., 1981; Miyajima and Bunag, 1986). Cardiopulmonary baroreceptors have been shown to participate in control of renal sodium and

water excretion, as well as renin release (Ferrari et al., 1984). This is due to the diminished inhibitory effect that cardiopulmonary baroreceptors have on renal sympathetic nerve activity, leading to net increase in sympathetic nerve activity (Ueno et al., 1988). However, the mechanism by which a high salt diet might sensitize arterial baroreceptors is not clear but could conceivably involve ionic or humoral adjustments (Mark, 1991). Thus, we thought it might be worthwhile to investigate the impairment of arterial baroreceptors in salt-induced hypertensive rats and the effect of dietary calcium on it from the same the perspective.

Methods

Animal preparation

Male in-bred Sprague-Dawley rats (90-110g) were randomly divided into four groups i.e.

control, salt-loaded, salt-loaded-calcium-fed and calcium-fed group, respectively. The control rats (NR) were fed normal rats chows containing 0.3% NaCl and 0.9% calcium. The salt-loaded group (SR) had a diet of 8% NaCl and 0.9% calcium, while the salt-loaded-calcium-fed (NaCaR) rats had an 8% NaCl and 2.5% calcium-fed rats (CaR) received a diet of 0.3% NaCl and 2.5% calcium. The salt and/or calcium diets were prepared from the normal rat feed by adding appropriate quantities of salt and/or calcium (as CaCl_2) to it. Measured food regimen and tap water for each group were provided ad libitum for 6 weeks. Urinary excretion was collected and measured. At the end of the period, the following experiments were carried out.

Blood pressure measurement

After the feeding period, rats from each group were randomly picked and anaesthetized by intraperitoneally injection of a mixture of 25% (w/v) urethane and 1% (w/v) alpha-chloralose at a dose of 5ml/kg body weight. The unconscious animal was fastened to the Small Operating Table (Bioscience, Sheerness, UK) with its temperature maintained at 37°C. The trachea, and right and left carotid arteries were exposed by blunt dissection. The trachea was cannulated for spontaneous respiration while threads were passed under the carotid arteries for easy access. The right femoral artery was also exposed and cannulated with catheter (3FG OD-0.7mm; length - 15cm, Portex Ltd, England) for blood pressure (BP) measurement. Heparin (500iu/kg-body weight) was infused into the cannulated artery immediately to prevent intravascular coagulation. The catheter was then connected to a Satham P23Dc blood pressure transducer (Hato Rey, Puerto Rico, USA) coupled to a grass polygraph (model 7D; Grass Instrument, Quincy, MA, USA). At the end of cannulation, the rats were allowed 30 minutes for the BP to stabilize before commencing the experiment. Phasic blood pressure were obtained as systolic and diastolic pressures and the mean arterial pressure (MAP) calculated from the relationship: $\text{MAP} = \text{diastolic pressure} + 1/3 \text{ pulse pressure}$.

Heart rate measurement

After fastening the animal to the small operating table, specially adapted platinum electrocardiogram (ECG) electrodes (No. 2185, Grass Instruments Co.) were placed on the limbs of the animal. These were passed to an ECG coupler (Model 796C; Grass Instruments Co.) unto another channel of the Grass Polygraph. The R-R interval was obtained from the ECG tracings, which was used to deduce the heart rate (HR). Thus, as the blood pressure measurement was recorded, the ECG was measured simultaneously.

Bilateral carotid occlusion test

Baroreceptor reflexes were investigated using the bilateral carotid occlusion (BCO) test. This was done by applying bulldog clips at a point on the right and left common carotid arteries about 10mm below the carotid bifurcation. BCO was carried out thrice in each rat at 10 minutes interval with each occlusion about 1 minute.

Data analysis and statistical significance

Data reported in this study is presented as mean \pm SEM. All data were analysed using a Macintosh Performa 5200CD Model computer loaded with Statview 4.5 statistical software package. One way analysis of variance was used to assess for difference among group. $P < 0.05$ was considered significant.

Results

Mean body weight, food consumption, salt-intake, calcium intake, water intake and urinary output in experimental groups

During the feeding period, salt-loading led to decrease in food consumption and body weight than SR. Salt intake was greatest ($P < 0.05$) in NaCaR, while NaCaR consumed significantly higher calcium than NR and SR (Table 1). In addition, salt-loading led to enhanced intake of water and increase in urinary output. SR consumed more ($P < 0.05$) water than NaCaR, while NaCaR excreted more ($P < 0.05$) urine than SR.

Table 1. Average body weight, food consumption, water intake and urinary output in experimental groups.

	NR	SR	NaCaR	CaR
Body weight (g)	147.3 ± 3.3	123.2 ± 1.5*	132.7 ± 2.0* [#]	143.0 ± 2.5 [#]
Food consumption (g/kg/day)	87.7 ± 2.7	45.2 ± 3.5*	56.5 ± 3.1* [#]	77.6 ± 2.1* [#]
Salt intake (mmol/Kg/day)	4.5 ± 0.1	48.1 ± 3.5*	62.4 ± 3.6* [#]	3.9 ± 0.1 [#]
Calcium intake (mmol/Kg/day)	7.1 ± 0.2	3.7 ± 0.3*	11.1 ± 0.4* [#]	16.0 ± 0.5* [#]
Water intake (ml/Kg/day)	101.8 ± 3.4	152.7 ± 4.9*	140.1 ± 4.5* [#]	104.0 ± 2.7 [#]
Urinary output (ml/Kg/day)	32. ± 1.2	87.7 ± 4.0*	106.2 ± 5.4* [#]	35.6 ± 1.4 [#]

Data are presented as mean ± SEM. *P<0.05 compared with control rats (ANOVA) while[#] P<0.05 compared with salt-loaded rats. NR, normal rats (control); SR, salt-loaded rats; NaCaR, salt-loaded-calcium-fed rats. N = 8 in each group.

Table 2: Blood Pressure (MAP) and Heart Rate (HR) changes due to baroreflex response to bilateral carotid occlusion test in experimental groups.

Blood Pressure				Heart Rate		
	Control MAP	Peak MAP	Change in MAP	Control HR	Peak HR	Change In HR
NR	90.2 ± 1.8	126.0 ± 4.8	35.8 ± 3.9	499 ± 27.1	535 ± 18.2	36 ± 10.5
SR	132.6 ± 2.7	188.8 ± 17.9	88.3 ± 13.0	438 ± 11.7	488 ± 23.0	50 ± 10.3
NaCaR	94.8 ± 2.6	129.4 ± 4.2	34.1 ± 4.0	414 ± 4.0	488 ± 11.5	63 ± 11.3
CaR	84.5 ± 1.9	105.1 ± 5.2	22.2 ± 4.1	540 ± 10.2	587 ± 20.2	48 ± 12.8

Data are presented as mean ± SEM. NR, normal rats (control); SR, salt-loaded rats; NaCaR, salt-loaded-calcium-fed rats, CaR, calcium-fed rats, n = 8 in each group.

Table 3: Multiple regression of the increases in mean arterial pressure (MAP) and heart rate (HR) induced by bilateral carotid occlusion with time

	NR	SR	NaCaR	CaR
MAP	-0.7 ± 0.3	0.6 ± 0.1*	0.5 ± 0.2	-0 ± 0.2
HR	0.22 ± 0.2	0.07 ± 0.5	0.3 ± 0.4	0.001 ± 0.3

Data are presented as mean ± SEM. [#]P<0.05 compared with other experimental groups. NR, normal rats (control); SR, salt-loaded rats; NaCaR, salt-loaded-calcium-fed rats; CaR, calcium-fed rats. n = 8 in each group.

Blood pressure and heart rate measurement

MAP of SR was significantly higher (132.6 ± 2.3 mmHg; P<0.05) than those of NR (90.1 ± 1.8 mmHg), CaR (84.5 ± 1.9 mmHg) or NaCaR (94.8 ± 2.5 mmHg). there was no

significant difference in the MAP of NR and NaCaR. However, salt-loading significantly (P<0.05) reduced the heart rate of NaCaR (379 ± 15 beats/min) compared with NR (418 ± 15 beats/min), while it did not affect the heart rate of SR (396 ± 12 beats/min). Calcium alone has no effect on heart rate (CaR, 413 ± 9 beats/min).

Bilateral carotid occlusion test

Figure 1 shows MAP response of experimental rats to BCO. There was significant increase in MAP due to BCO in all experimental groups, which was greatest ($P < 0.05$) in SR (Table 2). The multiple regressions of the slopes from the increase in MAP induced by BCO with time were significantly greater in SR (Table 3). Although, there was enhanced HR response due to BCO in all experimental groups, these were not regular. Hence, there was no significant difference in the multiple regression of their slopes (Table 3). Calculating baroreflex sensitivity from the ratio of $\Delta HR / \Delta MAP$ showed that salt-loaded rats had the lowest ($P < 0.05$) baroreflex sensitivity in NaCaR and CaR compared with NR.

Discussion

The results of the present study show that salt loading increased the arterial pressure of normotensive rats, while the elevation of arterial pressure was prevented by concurrent dietary calcium supplement in salt-loaded-calcium-fed rats. These observations confirmed reports that salt-loading may lead to hypertension (Adegunloye and Sofola, 1997; Hirawa et al, 1999; Quaschnig et al, 2001) while calcium supplement prevents the hypertensive effect of salt-loading (McCarron, 1989; Oparil et al, 1991). Although salt loading had no effect on heart rate, the antihypertensive effect of dietary calcium seems to be compensated with reflex bradycardia as reflected in the decrease of heart rate by salt-loaded-calcium-fed rats compared with controls. Earlier study (Adegunloye and Sofola, 1997) suggested increase in heart rate of salt-loaded rats compared with salt-loaded-calcium-fed and calcium -fed rats but similar heart rates with control.

Salt loading in this study led to decrease in food consumption but enhanced water intake, which was demonstrated by decreased body weight in salt-loaded and salt-loaded-calcium-fed rats compared with control. Thus, loss of weight in these rats may be attributed to large water consumption at the expense of food intake. This indicates that the thirst centre overrides those of hunger during salt loading. Brum et al (1991) reported the stimulation of the thirst centre in the brain of salt-loaded rats. This probably might be due to the mediating action of angiotensin II, which has been reported as acting on the central nervous system to stimulate drinking (McCarron et al, 1985; Jackson et al, 2000). The fact that salt-loaded-calcium -fed

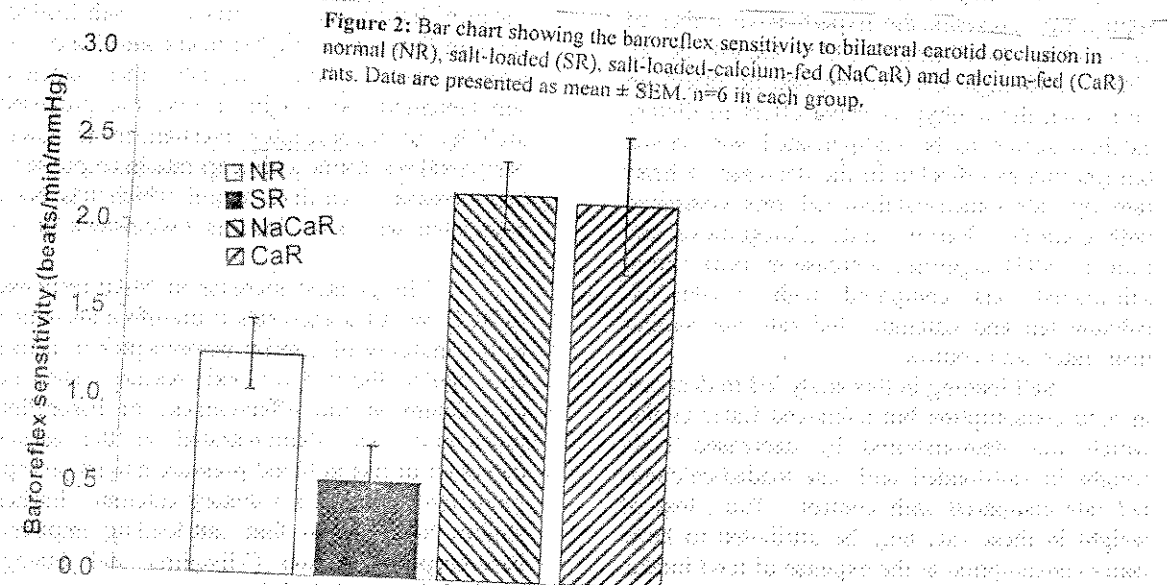
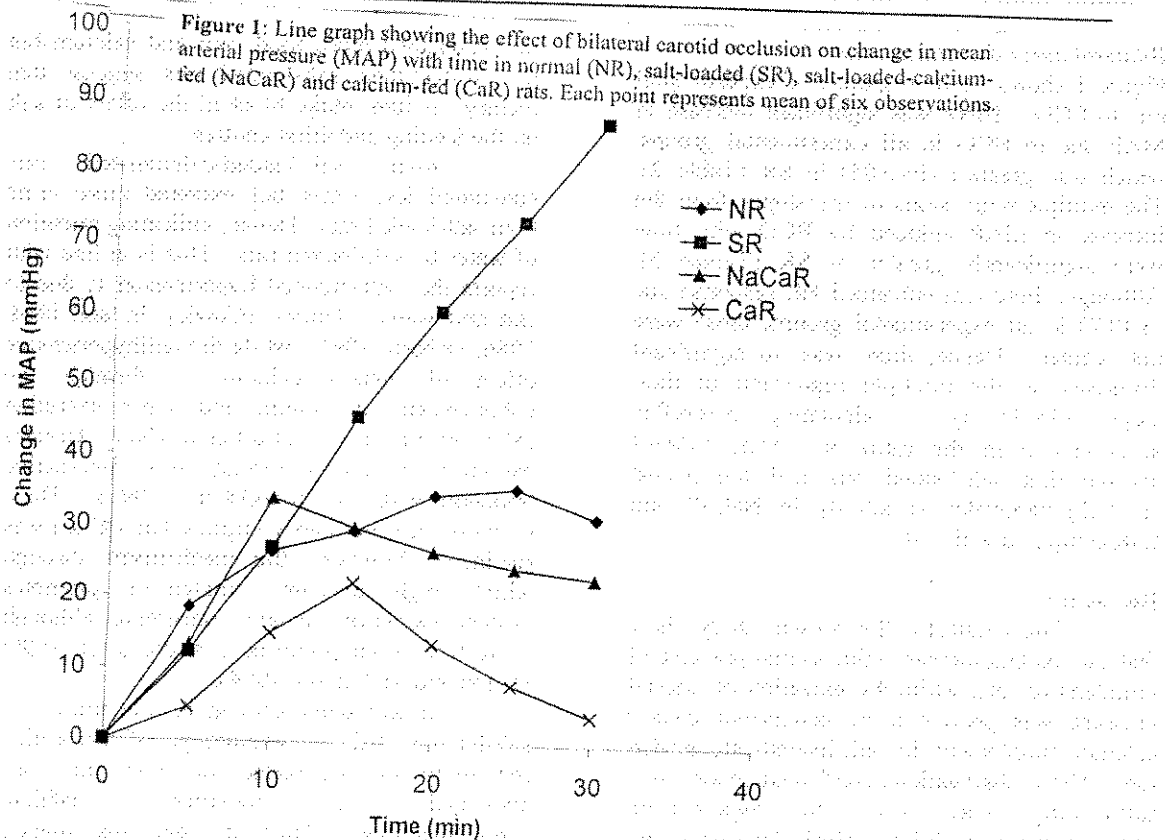
rats consumed more food, salt and calcium but less water than salt-loaded rats suggest that dietary calcium intake blocked the effect of salt on the feeding and thirst centres.

Also, salt-loaded-calcium-fed rats consumed less water but excreted more urine than salt-loaded rats. Hence, indicating retention of water by salt-loaded rats. This is in line with reports that salt-induced hypertension is due to salt and water retention (Cowley Jr. and Liard, 1986; Osborn, 1991), while the antihypertensive effect of dietary calcium is through the enhancement of sodium and water excretion (McCarron et al, 1985; Luft et al, 1986). Urinary excretion of water is through secondary active transport with sodium (Osborn, 1991). Thus, retention of water partly implies that sodium was retained, however, the mechanisms through which high calcium supplement promotes urinary excretion remains uncertain, although some have been proposed (Lahera et al, 1990; Hatton and McCarron, 1994).

It was demonstrated in this study that salt-loading led to attenuation of baroreflex sensitivity in salt-loaded rats but this was abolished by concurrent calcium supplementation. Thus, it seems that dietary calcium improves baroreflex sensitivity in rats because baroreflex sensitivity in salt-loaded-calcium-fed rats was higher than control and salt-loaded rats. This suggests that calcium supplemented diet might reverse the proposed inability of baroreceptor mechanism to lower peripheral resistance and heart rate in response to the increase of cardiac output which has been associated with salt-loading (Weinstock et al, 1996).

The greatest increase in MAP response to BCO by salt-loaded rats is therefore attributed to the inability of arterial baroreceptor to detect and regulate the rise in blood pressure. The role of calcium in the effectiveness of baroreflex mechanism was demonstrated in the earlier detection of rise in blood pressure and its prompt reduction by rats fed a dietary calcium. Earlier studies have shown that salt-loading impaired baroreceptor function (Miyajima and Bunag, 1987; Sofola et al, 1991).

In conclusion, the results of this study indicate that dietary calcium supplement enhanced baroreflex sensitivity in salt-loaded rats to prevent their hypertensive effect. This was associated with the prevention of enhanced thirst centre and inhibited renal function resulting in water retention by salt loading.



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REFERENCES

- Adegunloye, B.J. and Sofola, O.A. (1997). High dietary calcium prevents salt-induced hypertension and modifies contractile responses in aortic rings of rats. *Nig. Q. J. Hosp. Med.* 7(3), 292-295.

- Brum, J. M., Trampusch, A. F. and Ferraio, C.M. (1991) Neurovascular mechanisms and sodium balance in the pathogenesis of hypertension. *Hypertension* 17(suppl 1), 1-45-1-5-51
- Cowley, A.W. Jr. and Liard, J.F. (1986). Relationships between body fluid volume and arterial pressure. *Fed. Proc.* 45, 2864-2870
- Ferriari, A., Gordan, F.J. and Mark, A.L. (1984). Impairment of cardiopulmonary baroreflexes in Dahl salt-sensitive rats fed low salt. *Am. J. Physiol.* 227, H119-H123
- Gordon, F.J. Matsuguchi, H. and Mark, A.L. (1981). Abnormal baroreflex control of heart rate in prehypertensive and hypertensive Dahl genetically salt-sensitive rats. *Hypertension* 3 (suppl1), 1-35 - 1-41.
- Hatton, D.C and McCarron, D. A. (1994) Dietary calcium and blood pressure in experimental model of hypertension: A review. *Hypertension* 23, 513-530
- Hirawa, N., Uehara, Y., Suzuki, T., Kawabata, Y., Numabe, A., Gomi, T., Ikeda, T., Kizuki, K. and Ometa, M. (1999) Regression of glomerular injury by kallikrein infusion in Dahl salt-sensitive rats is a bradykinin B₂-receptor-mediated event. *Nephron* 81(2), 183-193
- Jackson, G., Gibbs, C.R., Davies, M.K. and Lip, G.Y.H. (2000): ABC of heart failure: Pathophysiology. *Br. Med. J.* 320, 167-170
- Lahera, V., Fisksen-Olsen, M.J. and Romero, J.C. (1990). Stimulation of renin releases by intrarenal calcium infusion. *Hypertension* 15 (suppl 1) 1-149-1152
- Luft, F.C., Aronoff, G.R., Sloan, R. S., Fineberg, N. S. Winberger, M.H. (1986) Short term augmented calcium intake has no effect on sodium homeostasis. *Clin. Pharmacol. Ther.* 39,414-419
- Mark, A.L. (1991). Sympathetic neural contribution to salt-induced hypertension in Dahl rats. *Hypertension* 17(suppl 1), 1-86-1-90
- McCarron, D. A., Lucas, P.A., Shreidman, R. J., Luceke, T. (1985) Blood pressure development of the spontaneously hypertensive rats after concurrent manipulation of dietary Ca²⁺ and Na⁺: relation to intestinal Ca²⁺ fluxes. *J. Clin. Invest.* 54, 1147-1154
- McCarron, D.A. (1989) Calcium metabolism and hypertension *Kidney Int.* 35, 717-736
- Miyajima, E. and Bunag, R.D. (1987). Exacerbation of central baroreflex impairment in Dahl rats on high-salt diets. *Am. J. Physiol.* 252, H402-H409
- Miyajima, E. and Bunag, R.D. (1986) Impaired sympathetic baroreflexes in prehypertensive Dahl salt-sensitive rats. *Clin. Exp. Theory Prac.* A8, 1049-1061
- Oparil, S., Chen, Y., Jin, H., Yang, R. and Wyss, J.M. (1991) Dietary Ca²⁺ prevents NaCl-sensitive hypertension in spontaneously hypertensive rats via sympatholytic and renal effects. *Am. J. Clin. Nutr.* 54, 2275-2365
- Osborn, J.L. (1991). Relation between sodium intake, renal function and the regulation of arterial pressure. *Hypertension* 17 (suppl 1), 1-91-1-96
- Quaschnig, T., D'Uscio, L.V., Shaw, S., Grone, H.J., Ruschitzka, F. and Luscher, T.F. (2001). Vasopeptidase inhibition restores renovascular endothelial dysfunction in salt-induced hypertension. *J. Am. Soc. Nephrol.* 12(11), 2280-2287
- Resnick, L.M. (1999). The role of dietary calcium in hypertension: a hierarchical overview. *Am. J. Hypertens.* 12 (1Pt 1), 99-112
- Saito, K., Sano H., Furuta, Y. and Fukuzaki, H. (1989) Effect of calcium on blood pressure response in salt-loaded borderline hypertensive patient *Hypertension* 13, 219-226
- Sofola, O.A., Dina, T.A., Egbe, P.E. and Owolabi, O.A. (1991) Dietary salt-loading enhances baroreflex sensitivity in Sprague-Dawley rats. *Nig. Physiol Sci.* 7, 95-99
- Ueno, Y., Mohara, O., Brosnihan, K.B. and Ferrario, C.M. (1988) Characteristics of hormonal and neurogenic mechanisms of DOC-induced hypertension *Hypertension* 11 (suppl 1), 1-172-1-177
- Weinstock, M., Gorodetsky, E. and Kalman, R. (1996) Renal denervation prevents sodium retention and hypertension in salt-sensitive rabbit with genetic baroreflex impairment *Clin. Sci. (Colch)* 90(4), 287-293

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