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Cardiovascular Response to Angiotensin II and Captopril in Normal and Diabetic Rats Loaded with Salt

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The results of the present study demonstrated that dietary salt loading in normal and diabetic rats increased the blood pressure, blood volume, plasma sodium level and sensitivity of cardiovascular responses to angiotensin II and Captopril. This probably suggests that salt loading interferes with the mechanism regulating blood pressure in salt loaded rats either by decreasing the secretion of vasodilators mediators, increasing the production of vasoconstrictors, increasing the sensitivity to vasoconstrictors and/or resistance of vascular smooth muscle to endothelial vasodilators. It was then concluded that the increase in the sensitivity to angiotensin II and hypervolaemia, probably caused the development and sustenance of hypertension observed in salt loading diabetic rats.

Key words: Diabetic, salt loading, angiotensin II, hypertension, captopril

INTRODUCTION

Diabetes mellitus is a constellation of anatomical and biochemical abnormalities resulting in disorder of carbohydrate metabolism (Collins and Dicarlo, 1995). It is a genetically determined chronic disorder of carbohydrate metabolism resulting in a clinical syndrome combining several pathological events into a common clinical picture. (Christlieb *et al.*, 1981). Both insulin-dependent diabetes mellitus (IDDM) and non -insulin-dependent diabetes mellitus (NIDDM) are associated with a higher prevalence of hypertension (Simonson, 1988; Weidmann *et al.*, 1985).

Hypertension is a complex pathophysiological state that manifests itself as chronically high blood pressure (BP) and is a major risk factor for many cardiovascular diseases such as stroke, heart failure, coronary artery diseases and progressive renovascular damage (Meneton *et al.*, 2005; Iwamoto, 2006). There is considerable clinical evidence that higher proportion of patient with borderline or definite hypertension later developed diabetes (Simonson, 1988). There is overwhelming evidence for an association between sodium and hypertension in patients with obesity, glucose intolerance and diabetes mellitus (Stern and Tuck, 1990; Jones, 2004; Khalil, 2006; Siani *et al.*, 2004).

Mechanisms that render the diabetic population more susceptible to hypertension remain unknown.

The mechanisms of hypertension in diabetes appear to be complex and may involve alterations in both reflex function and in the vascular responses to hormonal stimuli (Siani *et al.*, 2004).

It is now well established that Angiotensin II plays an important role in the regulation of sodium excretion, intravascular fluid volume, vascular resistance and blood volume (Kosachunhanun *et al.*, 2003; Lavoie *et al.*, 2004), although the effects of different levels of salt intake on this hormone is much less clear (Stern and Tuck, 1990; Sofola *et al.*, 2002). In diabetics, studies may indicated that rennin production and/or secretion could be altered because of disturbances in neuroendocrine and other regulatory control mechanisms. Decreased sympathetic nervous activity (Dury *et al.*, 1984), impaired responsiveness of rennin release, renal prostaglandin deficiency, sodium retention and hypertension (Sofola *et al.*, 2002) are diabetic complications that tend to reduce rennin secretion (Collins and Dicarlo, 1995).

Adigun and Akinyanju (1991) demonstrated that the circulating angiotensin in salt loaded rat was very low, it was suggested that increase in blood pressure observed in salt loading rats may be influenced by the increase in sensitivity to circulating angiotensin.

However, the report of Tuck *et al.* (1991) showed that short-term salt loading in hypertensive diabetic patients increased the mean arterial pressure levels (MAP) and enhanced pressure responses to angiotensin during sodium intake. This study was performed in hypertensive diabetic patients and without careful control of salt intake.

However, there appears to be no reports so far on the effect of long-term dietary sodium intake on cardiovascular responses to angiotensin in uncomplicated diabetes mellitus.

The present study was therefore designed to investigate the effect of salt loading on cardiovascular responses to angiotensin and captopril.

MATERIALS AND METHODS

Sprague Dawley rats aged 6 weeks weighing 200 to 250 g obtained from the Animal Centre of the College of Medicine, Lagos State University, were used in the study. The rats were allowed to acclimatize in environmentally controlled quarters with temperature maintained at 30°C, relative humidity 35% and lighting with 12-12h light /dark cycles. Standard laboratory chow (Live stock feeds in Ikeja, Lagos Nigeria) and drinking water were provided *ad libitum*.

Induction of diabetes: Diabetes was induced with alloxan monohydrate injected intraperitoneally at a dose of 50 mg kg⁻¹ body weight daily for three consecutive days. The condition was confirmed by the analysis of fasting blood glucose as described by Verley (1964).

Preparation of special diet: Sodium loaded diet was prepared from the standard pellet containing 0.3% NaCl (supplied by livestock feeds, Ikeja, Lagos).

The high salt chow was prepared by mixing 76 g of sodium chloride with 924 g of cow (8% NaCl); the rats were fed on these diets for 8 weeks as previously reported (Adigun and Akinyanju, 1991)

Experimental design: Four groups of rats with 18 rats in each group were housed in a cage at a room temperature of about 28°C for eight weeks. The first group of rats was kept on normal diet and normal drinking water, referred to as Normal Rat (NR). Group II was salt loaded with diet containing 8% NaCl and normal drinking water and referred to as salt loaded rats (SR). Group III consisted of diabetic rats on normal diet and normal drinking water referred to as Diabetic Rats (DR). Group IV consisted of diabetic rats salt loaded with diet containing 8% NaCl and normal drinking water and

referred to as diabetic salt loaded rats (DSR). Each of the three groups received the treatment for 8 weeks. All experimental procedures complied with the Animals (Scientific Procedures) Act, 1986.

Experimental procedures: On the day of the experiment, the rats were weighed and anaesthetized with a 50:50 mixture of 25% Urethane and 1% alpha chloralose at a dose 5 mL kg⁻¹ body weight. Each rat was dissected to expose the trachea. The trachea was cannulated for clear airway and spontaneous respiration. The femoral artery and vein were exposed and cannulated using polythene catheters (3FG outer diameter 0.7 mm and 15 cm long) filled with heparinized 0.9% (w/v) NaCl solution. The cannulated artery was used for blood pressure recording while the vein was used for injection of drugs. Thirty minutes after all cannulations were completed and the blood pressure had stabilized, control Blood Pressure (BP) and Heart Rate (HR) were taken before drug administration (Salahdeen *et al.*, 2004).

Following the drug injection, the responses of heart rate and blood pressure were then determined, the mean blood pressure was measured and determined from diastolic blood pressure plus one third of pulse pressure (Salahdeen *et al.*, 2004). Cumulative concentration response to angiotensin II and captopril was determined after each injection. The recordings were allowed to approach or reach the pre-injection level before another dose was administered; this usually last 10-20 min.

Arterial blood pressure was measured by means of a Statham pressure gauge transducer connected to a grass 7D polygraph recorder (Grass instruments Ltd., Quincy, Mass USA). The heart rate was computed from the blood pressure tracing.

Determination of blood volume: Plasma volume and hence calculated blood volume was estimated by dye-dilution (Evans Blue) method as described by Adigun and Akinyanjuola (1991). The experiments were performed on the anaesthetized rats as follows: Four standard solutions of 12, 20, 28, 36 µg mL⁻¹ were prepared from the original stock solution of (500 µg mL⁻¹ of the Evans blue) solution. This was injected at zero time. At precisely 10 min after, 1.5 mL of blood was rapidly withdrawn into heparinized syringe from the carotid artery. The sample was then immediately centrifuged (3000 rev/min) for 10 min. The absorbance of the concentration of Evans blue dye in the test and standard sample was then measured with spectrophotometer (zeiss, spectral photometer pm 2A) at a wave length 610 nm. Haematocrit (Hct) of the blood

sample was also determined using micro-capillary tubes (Hawksley UK). The blood volume was later calculated from plasma volume and haematocrit.

Determination of Plasma Na⁺ and K⁺ levels: The Boiling (1964) method was used. The plasma from each blood sample and was then analyzed for sodium and potassium concentrations using flame photometry (corning 400 flame photometer-corning Medical Equipments Ltd.). Fresh standards containing 160 mmol L⁻¹ of Na⁺ and 8 mmol of K⁺ were used to standardize the photometer for estimation of the two electrolytes. Deionized distilled water was used as blank and as diluents for plasma.

Blood glucose measurement: Blood glucose was determined by the glucose oxidase method as modified by Barham and Trinder (1972).

Statistical analysis: The data are presented as mean±SEM. One way (ANOVA) and the paired student's t-test were used to assess statistical significant difference and a probability of p<0.05 was considered significant.

RESULTS

Effect of sodium chloride on body weight, blood volume, electrolytes and glucose levels: Figure 1 shows that the body weight of animals in four groups showed no significant difference on the first day of the experiment. At the end of 8 weeks of receiving special diet the body weight of rats in Normal Group (NR) showed no significant difference. The Diabetic Rats (DR) showed a significant fall in body weight (p<0.001). The salt loaded (SR) and diabetic salt loaded rats (DSR) showed significant increases in body weight during the 8 weeks (p<0.001) though the increase was higher in the former (p<0.001). Figure 2 shows the effect of salt loading on blood volume. Blood volume increases by 24% in salt loaded rat (SR) compare to control (NR) and by 29% in diabetic salt loaded rat (DSR) compare to Diabetic Rats (DR) (p<0.001). In other word the blood volume increased significantly in both groups. Figure 3 shows the effect of salt loading on plasma level of sodium and potassium. Salt loading caused a significant increase in the plasma level of Na⁺ by 15% and significantly decreases plasma level of K⁺ by 41% (p<0.05) and (p<0.001), respectively in both salt loaded (SR) and Diabetic Salt loaded (DSR) rats.

Figure 4 shows the effect of salt loading on glucose levels. Salt loading increases the glucose level by 10% in salt loaded (SR) compare to control (NR) rats. While in

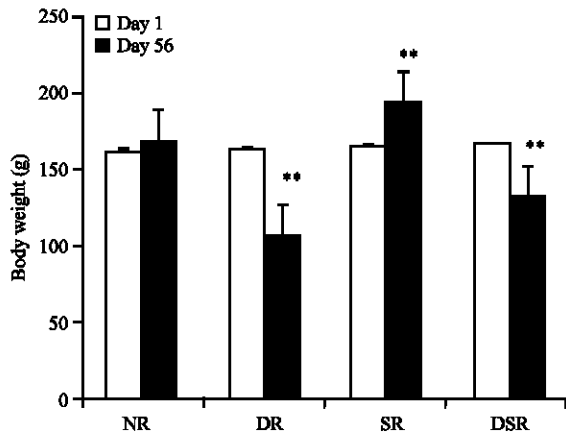


Fig. 1: Effects of dietary sodium chloride on body weight in normal (NR) Diabetic (DR) salt loaded (SR) and Diabetic salt loaded (DSR) Rats (n = 8) (**p<0.001)

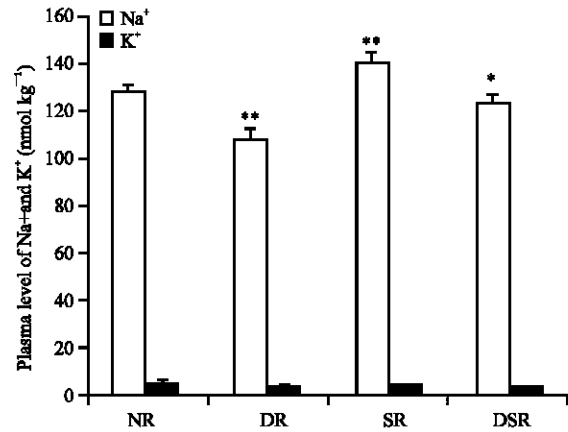


Fig. 3: Effect of dietary sodium chloride on plasma level of Na⁺ and K⁺ in normal, (NR) diabetic (DR) salt loaded (SR) and diabetic salt loaded (DSR) rats (n = 8) *p<0.05, **p<0.001

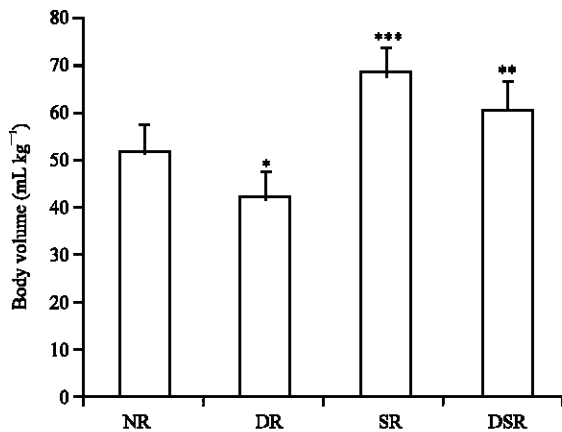


Fig. 2: Effect of dietary sodium chloride on blood volume in normal (NR), Diabetic (DR) salt loaded (SR) and diabetic salt loaded rats (n = 8) *p<0.05, **p<0.01, ***p<0.001

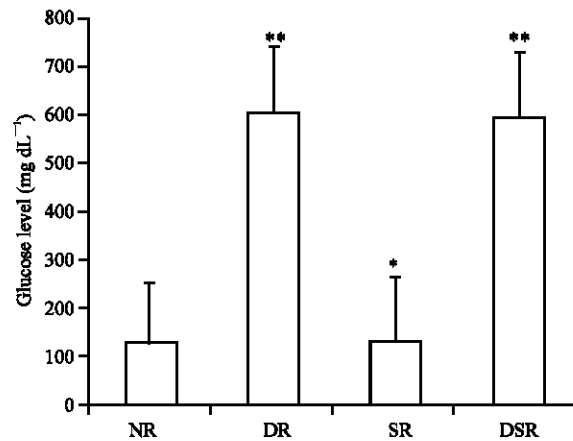


Fig. 4: Effect of dietary sodium chloride on plasma glucose level in normal (NR), Diabetic (DR), salt loaded (SR) and diabetic salt loaded (DSR) rats (n = 8) *p<0.05, **p<0.001

diabetic salt loaded (DSR) the glucose increased by 2.5% compare to diabetic (DR) rats. The glucose level in control and diabetic rats are 121.1±2.1 and 606.3±5.4 mg dL⁻¹ respectively. This represents a 40.4% increase compare to control value (p<0.001).

Effect of sodium chloride on systolic, diastolic, mean arterial blood pressure and heart rate: The effect of salt loading on blood pressure is shown in Fig. 5. Salt loading increases systolic pressure by 25 and 37%, diastolic pressure by 36 and 34% and mean arterial blood pressure by 25 and 28% in both salt loaded (SR) and diabetic salt loaded (DSR) (p<0.001). There was no significant increase in the blood pressure of both salt loaded

and diabetic salt loaded rats. However, in the Diabetic Rats (DR) the blood pressure shows a significant decrease of about 20% in systolic, diastolic and mean arterial blood pressure compare to the control (p<0.01).

Figure 6 shows the effect of salt loading on heart rate. Salt loading increased heart rate significantly in salt loaded rats and insignificantly in diabetic salt loaded rats.

Effect of sodium chloride on cardiovascular responses to angiotensin II and captopril: Figure 7 shows the effect of salt loading on cardiovascular responses to angiotensin II. Salt loading potentiates the effect of

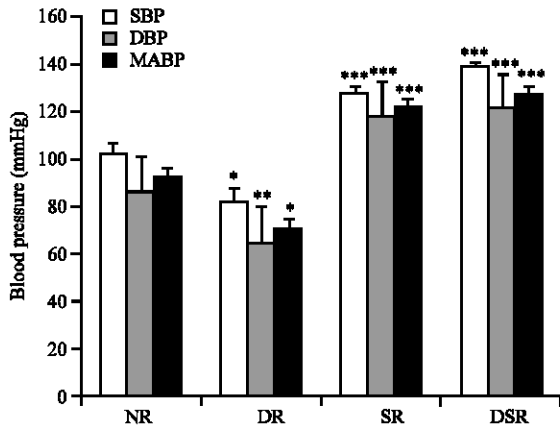


Fig. 5: Effect of dietary sodium chloride on systolic, diastolic, mean arterial blood pressure in normal (NR) and diabetic (DR) salt loaded (SR) and diabetic salt loaded (DSR) rats. (n = 8) *p<0.05, **p<0.01, ***p<0.001

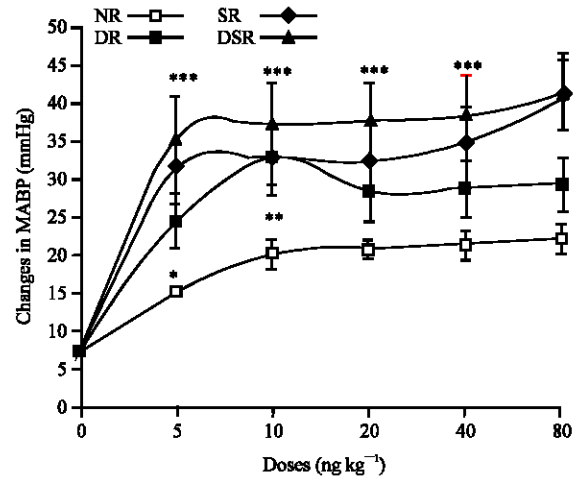


Fig. 7: Effect of dietary sodium chloride on maximum increase in mean arterial blood pressure induced by angiotensin II in normal (NR), diabetic (DR), salt loaded (SR) and diabetic salt loaded (DSR) rats (n = 8) *p<0.05, **p<0.01, ***p<0.001

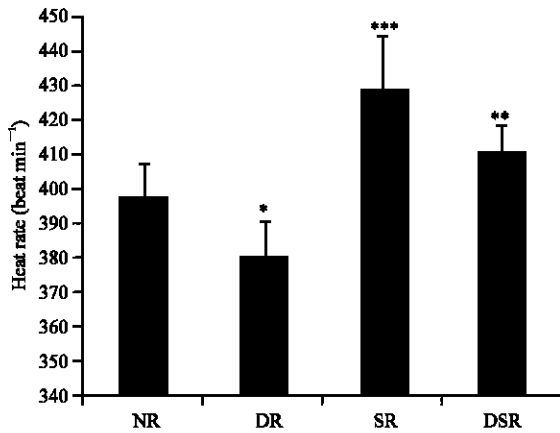


Fig. 6: Effect of dietary sodium chloride on heart rate in normal (NR), diabetic (DR), salt loaded (SR) and diabetic salt loaded (DSR) rats (n = 8) *p<0.05, **p<0.01, ***p<0.001

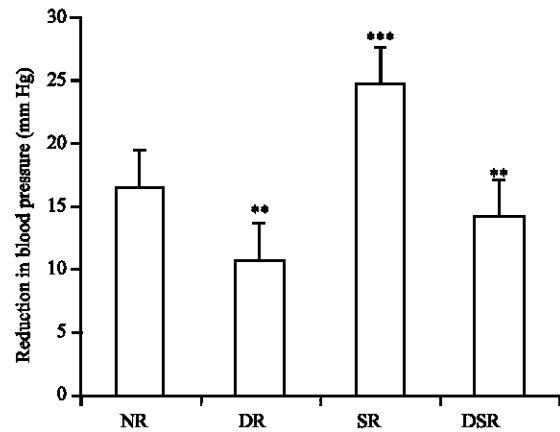


Fig. 8: Effect of dietary sodium chloride on Max. Reduction in MABP induced by captopril in normal (NR), diabetic (DR) Salt loaded (SR) and diabetic salt loaded rats. (n = 8) p<0.05, *** p<0.001

angiotensin on blood pressure in all the groups (p<0.001), the increase was higher in salt loaded diabetic (DSR) than in salt loaded (SR) rats (p<0.001). The changes in heart rate induced by angiotensin was not of significant difference in all groups.

Figure 8 shows that captopril caused a significant reduction in mean arterial blood pressure produced by salt loading. In fact, the blood pressure decreased by about 65% in salt loaded (SR) rats compare to control (p<0.001). This decrease was not significantly different from control in salt loaded diabetic rats.

DISCUSSION

The significant decrease in body weight in rats after injection of alloxan observed in this study agrees with some of the previous reports (Rerup, 1970; Zhao *et al.*, 1999). We further demonstrated that alloxan induced diabetes was associated with hypotension. Early studies provided conflicting results concerning blood pressure in diabetic animals, in which blood pressure was reduced

(Chrislieb, 1971), unchanged (McKinley and Johnson, 2004) and even enhanced (Tuck, 1991). Previous studies suggested that diabetes mellitus is often associated with hypertension as opposed the hypotension observed in this study (Dupree and Meyer, 1980). The discrepancies could be related to the toxic effect of alloxan, metabolic changes induced by diabetes mellitus and other confounding factors such as obesity, age, sex/race and genetic factors may as well contribute towards this discrepancies (Kosachunhanun *et al.*, 2003).

The present study also indicated that salt loading increases the blood pressure, blood volume, sodium plasma levels and enhance vasopressor response to angiotensin in normal and diabetic rats. That dietary sodium chloride could lead to hypertension in salt sensitive or genetically susceptible animals have been previously documented (Adigun and Akinyanju, 1991; Tuck, 1991).

Mechanisms responsible for the increase in blood pressure and response to angiotensin after salt loading in diabetes is unknown. The reduction in the blood pressure by captopril (an angiotensin converting enzyme inhibitor) in the present study confirmed the previous reports that hypervolemia and activation of renin-angiotensin system may be the possible mechanisms (Salazar and Llina, 1996). However, the balance experimental evidence suggested that in diabetes, high glucose level reduces the availability of nitric oxide (NO) and increasing oxidative destruction of the molecule (Cowley *et al.*, 2003; Herrera and Garvin, 2005). Hyperglycemia may activate the production of Protein Kinase C (PKC), which lead to production of advanced glycation end products. This may disrupt the vascular NO system (Zewde *et al.*, 2004; Takenaka *et al.*, 1993). Insulin is known to stimulate NO production and with diminished insulin action this may impede the positive effect of insulin on vascular NO release and thus promotes sodium retention and volume expansion (Tian *et al.*, 2003; Salazar and Llina, 1993). Also deficiency of nitric oxide synthetase has been reported in salt loaded animals (Rudd *et al.*, 1999; Zhu *et al.*, 2004).

Previous reports indicated that circulating Angiotensin II levels are not different from normal in diabetic (Lahera, 1991). When salt intake increases, angiotensin II levels fall and the sympathoexcitatory actions of angiotensin II are lost, thus increases the angiotensin sensitivity. Although plasma level of angiotensin and Nitric oxide synthesis (NOs) are not measured in this study, experimental evidence suggested that Angiotensin interact with NO in the regulation of glomerular vascular resistance (Johnson *et al.*, 2005).

Lahera *et al.* (1991) first observed that the acute and progressive inhibition of NO in rats obtained by the successive infusion of NO synthetase inhibitor L-NAME, produced significant decrements in urine volume, urine sodium excretions, renal blood flow and glomerular filtration rate (GFR) before the elevation of blood pressure was detected. This observation is consistent with the idea that the effects on NO are continuously countervailed by the vasoconstrictor effect of angiotensin II (Johnson *et al.*, 2005).

Thus, the increase in the blood pressure observed in this study may be due to increase or decrease in endogenous angiotensin, whose antinatriuretic and vasoconstrictor effects are left unbalanced as a result of progressive inhibition of NO. It is therefore hypothesized that if NO synthesis is reduced, none of the various natriuretic hormones in the body is to off set the antinatriuretic effects of the decrease NO synthesis. Thus, an increase in blood pressure is required to maintain sodium balance (Khalil, 2006; Zhu *et al.*, 2004).

Therefore the hypertensions induced by sodium loading in diabetic rats in this study are probably similar to those in non-diabetics. However, enhanced vasopressor responses to angiotensin and hypervolemia contribute more to the sodium chloride induced hypertension.

Although much remains to be discovered about how salt retention is translated into central inhibition of nitric oxide that mediate changes in the angiotensin receptors activity, including the following: cellular/molecular mechanism by which sodium receptors affect and sustained changes in NO synthesis and how the signals from these multiple and diverse receptors interact.

Given the deleterious effects of salt on the body, it is imperative to seek answer to these fundamental questions.

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