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SALT INTAKE AND MEAN ARTERIAL BLOOD PRESSURE IN RABBITS.

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ABSTRACT
10 New Zealand white/Chinchilla crossbred rabbits, weighing between 1.5 – 1.8 kg were divided into two groups of five animals each. They were all fed standard rabbit chow (150g/rabbit/day). Group I or control rabbits were given water *ad libitum* while group II rabbits were given 1.2% saline drink *ad libitum* in place of water. The study spanned for 7 weeks, at the end of which it was discovered that administering 1.2% saline to rabbits for 7 weeks could not cause a significant elevation in their blood pressure. Also there was no significant difference ($p<0.05$) in heart rates and organ weights (kidney and heart) between groups.

Keywords: Saline, mean arterial blood pressure, rabbits

INTRODUCTION

Dietary salt loading with 8% sodium chloride $^{w}/_{w}$ (Miyajima, 1985) or 1.2% saline drink (Dina, 1986) has been shown to cause an elevation of blood pressure in rats. Dietary salt intake has also been shown from epidemiological studies to be related to the incidence of high blood pressure in some communities (Truswell, 1985; Sofola, 1992). The role of salt restriction in the diet or potassium supplementation have been investigated and have shown to ameliorate the levels of blood pressure in hypertension (Kawano, 1996) especially in older hypertensives (Cappuccino, 1997).

In adults, a diastolic pressure below 85 mmHg is considered to be normal; one between 85-89 mmHg is high normal; one of 90-99 mmHg represents stage 1 or mild hypertension; one of 100-109 mmHg represents stage 2 or moderate hypertension; and distolic blood pressure of ≥ 110 mmHg represents stage 3 or severe hypertension.

A systolic blood pressure below 130 mmHg indicates normal blood pressure; one between 130-139 mmHg indicates high normal; one between 140 – 159 mmHg indicates stage 1 or mild hypertension; one between 160-179 mmHg indicates stage 2 or moderate hypertension; and systolic blood pressure of ≥ 180 mmHg indicates stage 3 or severe hypertension (Gordon, 2001).

Hypertension is one of the most common diseases in the world, affecting an estimated 20% of the adult population (Prescott-Clarke, 1998). It is associated with marked morbidity and mortality and places a high burden on health care systems. Hypertension is the most important single, modifiable risk factor for coronary artery disease, stroke and end-stage renal disease (Martin, 2007). However, early recognition and implementation of effective pharmacological and non-pharmacological therapies allows for early modification of cardiovascular risk (Martin, 2007).

Epidemiological studies have shown a clear association between obesity, salt, alcohol intake and the development of hypertension. (Stamler, 1978; Mulrow, 2000). The association between salt intake and hypertension is more pronounced in the older population (Martin, 2007). There is a significant relationship between drinking more than three units of alcohol per day and the development of hypertension, independent of weight and salt intake (Marmot *et al.*, 1994).

With regards to public health, hypertension is a major risk factor in premature death and disability from heart attack, heart failure, stroke and many other afflictions (Chobanian *et al*., 2003; Kaplan, 2002). In a small fraction of cases, the hypertension is due to specific causes such as renal vascular disease or excessive secretion of aldosterone (primary aldosteronism) or catecholamines (Pheochromocytoma). The majority (approximately 90%) of patients, however, have elevated blood pressure of unknown cause; hence the term, primary or essential hypertension. The immediate cause for the elevated blood pressure in nearly all chronic hypertensive persons is exessive narrowing of the small (resistance) arteries (Mordecai *et al*., 2006).

Experimental studies have shown that high salt diet results in elevated blood pressure in animals such as rats e.g. Dahl Salt Sensitive (DS) rats, Spontaneously Hypertensive or SH rats, Sprague Dawley rats (Miyajima & Bunag, 1985; Obiefuna *et al*., 1991; Sofola *et al*., 2002), dogs (Vogel, 1966; Hainsworth, 2003) and chimpanzees (Denton *et al*., 1995). Chronic ingestion of excess salt produces hypertension in rats, which mimics human hypertension morphologically (Dahl, 1960). High salt intake hypertension has been produced in rats, rabbits and chicks by replacing drinking water with 1-2% sodium chloride for 9-12 months (Boura, 1964; Rathod *et al*., 1997).

The aim of this research is to ascertain whether administering 1.2% saline drink to rabbits for 7 weeks will elevate their mean arterial blood pressure as it does in rats so that they can be employed as hypertensive models in cardiovascular studies.

MATERIALS AND METHODS

10 New Zealand white/Chinchilla crossbred male rabbits aged 6 months-1 year were purchased from the Rabbit unit of the Teaching and Research Farm, University of Agriculture, Abeokuta. They were divided into 2 groups of 5 rabbits each and housed in clean hutches in the rabbit house. Their weight range was between 1.5-1.8 kg.

Group I or control group (CR) were maintained on commercially procured feed [Guinea growers mash (product of Bendel Feed and Flour Mills, Ltd; Ewu, Nigeria)] and were given 150g of feed per rabbit per day. They were given water *ad libitum*.

Group II rabbit (SR), were fed as group I rabbits but were given 1.2% saline (constituted by adding 1.2 g of salt into 100ml of clean water) drink *ad libitum*.

These protocols were sustained for 7 weeks after which the blood pressures of the rabbits were measured.

Animal preparation for blood pressure measurement: Using 25% urethane and 1% chloralose as the anaesthetic agent with 10% lignocaine and under aseptic conditions, an incision was made at the groin of the rabbits after the skin around the incision site had been shaved and cleaned. A blunt dissection was performed to isolate the femoral artery from the surrounding

tissues. The femoral artery was identified as a reddish and pulsatile vessel.

The vessel was tied proximately and clamped with a bull-dog clip distally. A small incision was made on the vessel near the distal end of the tie and the canula was introduced retrogradely into the vessel. The canula was filled with 0.01% heparinised saline prior to insertion to avoid blood clots. The vessel was then tied unto the canula. The bull-dog clip was removed and the tie was secured.

Blood pressure and heart rate measurement: Arterial blood pressure and heart rate were measured through the arterial canula connected to a strain-gauge pressure transducer (Statham strain-gauge pressure transducer) connected to a recorder (Grass polygraph, Model 70, Grass instrument, Quincy, MA).

The heart rate was calculated from the number of arterial pulses. Mean arterial blood pressure (MABP) was then calculated from the systolic and diastolic blood pressure using the following formular:

MABP = Diastolic pressure + $^1/_3$ Pulse pressure

Pulse pressures = Systolic blood pressure – Diastolic blood pressure (Cummingham & Klein, 2007)

Data analyses: The data were expressed as mean ± SEM (standard error of mean) and differences between mean values of the control and salt + losartan, and control and salt treated groups recordings were assessed by the use of the unpaired two-tailed Student's t-test. The accepted significant level was $p < 0.05$ in the analysis of all data.

RESULTS

Mean arterial blood pressure (MABP) was 65.3 ± 2.5 mmHg (n=5) in the CR and 69.9 ± 3.2 mmHg (n=5) in the SR. MABP seemed be higher in the SR compared with the CR but difference was not significant ($p > 0.05$). Control heart rate was 122.4 ± 0.87 beats/min and salt loaded heart rate, 114.2 ± 2.35 beats/min). There was significant difference when both groups were compared ($p < 0.05$) (Table 1).

Kidney weight was 5.0 ± 0.23g (n=5) in the control rabbits and 5.3 ± 0.30g (n=5) in the salt loaded rabbits. Kidney weight tended to increase in the salt loaded group compared with the control but there was no significant difference ($p > 0.05$) (Table 1).

Heart weight was 4.2 ± 0.07g (n=5) in the control rabbits and 4.6 ± 0.07g (n=5) in the salt loaded rabbits. There was significant difference when both groups were compared ($p < 0.05$) (Table 1).

TABLE 1. THE MEAN VALUES ± SEM OF ARTERIAL BLOOD PRESSURES, HEART RATES, KIDNEY WEIGHTS AND HEART WEIGHT OF RABBITS IN CONTROL AND SALT GROUPS (n = 5).

Parameter	Control Group	Salt Group
Mean Arterial BP (mmHg)	*65.32 ± 4.53	*69.92 ± 5.63
HR (beats/minute)	122.4 ± 0.87	114.2 ± 2.35
Kidney weight (g)	5.02 ± 0.15	5.34 ± 0.20
Heart weight (g)	*4.16 ± 0.07	*4.58 ± 0.07

*Significant values ($p < 0.05$)

DISCUSSION

Dietary salt loading to various strains of rats is known to result in increases in arterial blood pressure. This effect is greatest in the genetically selected Dahl salt-sensitive animals (Dahl *et al.,* 1962) but it also occurs in other animals including Sprague-Dawley rats (Torii, 1980; Miyajima & Bunag, 1985; Obiefuna *et al.,* 1991; Giardina *et al.,* 2000 & Kagota *et al.,* 2001). Experimental studies have shown that high salt diet results in elevated blood pressure in animals such as rats e.g. Dahl Salt Sensitive (DS) rats, spontaneously hypertensive or SH rats, Sprague Dawley rats (Miyajima & Bunag, 1985; Obiefuna *et al.,* 1991; Sofola *et al.,* 2002), dogs (Vogel, 1966; Hainsworth, 2003) and chimpanzees (Denton *et al.,* 1995). Chronic ingestion of excess salt produces hypertension in rats, which mimics human hypertension morphologically (Dahl, 1960). High salt intake hypertension has been produced in rats, rabbits and chicks by replacing drinking water with 1-2% sodium chloride for 9-12 months (Boura 1964; Rathod *et al.,* 1997). 10% NaCl has also been administered intravenously for 10 days to induce hypertension in rabbits (Ryuzaki *et al.,* 1991).

The results obtained from this study indicated that blood pressure does not increase significantly when rabbits are given 1.2% saline drink for 7 weeks (acute administration) (Table 1). This is in contrast to high salt in the diet that has been shown to elevate blood pressure (Miyajima, 1985; Turswell, 1985; Dina, 1986; Sofola, 1992; Kawano, 1996). In this study, the rabbits were given 1.2% saline drink for 7 weeks, but it was discovered at the end of the study that, the rabbits did not become significantly hypertensive. This may be due to the short duration (7 weeks) with which the rabbits were given the saline drink, compared with the study of Boura and Green (Boura & Green, 1969). In addition the level of salt intake was less than the 8% used in previously documented experiments on high salt (Dahl, 1962; Miyajima & Bunag, 1985; Obiefuna *et al*., 1991 & Sofola et al., 2002). It was obvious from the present results that the rabbits were becoming hypertensive, but the hypertension was not significant ($p > 0.05$).

There were postulations on the probable mechanism of salt loading on cardiovascular and renal parameters. One of these mechanism is the effect on a circulating inhibitor (i.e. ouabain-like or digitalis-like compound) of the Na-K-ATPase pump which is inhibited by salt loading (Haddy & Overbeck, 1976; Blaustein, 1977; Borghi *et al*., 1990). It could be possible that this was responsible for the increased vascular tone.

It is known that endogenous ouabain, like its plant-derived counterpart, is a Na^+ pump inhibitor that has cardiotonic and vasotonic actions; indeed, the effects of endogenous ouabain and exogenous ouabain are indistinguishable (Bova *et al*., 1991; Hamlyn *et al*., 1991).

Initial studies by Hamlyn and his team revealed that endogenous ouabain did not come from the diet and that it was synthesized and secreted by the adrenal cortex (Hamlyn *et al*., 1991). There are reports that endogenous ouabain also may be produced in the brain (Haber & Haupert, 1987) and that elevated cerebrospinal fluid levels (Haddy & Overbeck, 1976) are associated with hypertension without an elevation in the circulating endogenous ouabain level (Huang *et al*., 2005).

The synthesis and secretion of endogenous ouabain like aldosterone, occurs in the adrenal glomerulosa cells (Laredo *et al*., 1995; Shah *et al*., 1998) and has been reported to be stimulated by chronic high salt intake (Manunta *et al*., 2006) and by ACTH (Laredo *et al*., 1994 and 1995).

Earlier studies have suggested a direct tissue effect of salt independent of its ability to raise blood pressure. In animal experiments, it has been observed ((Yuan & Leenen, 1991, Frohlich *et al*., 1993) that high dietary salt induced left ventricular hypertrophy (LVH), without any significant elevation of blood pressure. Clinical studies by Schneider *et al*., (1988) showed that high salt intake is a powerful independent determinant of left

ventricular hypertrophy. Left ventricular hypertrophy is the principal cardiac end organ damage which results from a persistently raised afterload (the force against which the heart muscle must work to overcome resistance to blood flow in the aorta and peripheral arteries). At the close of this study, organ weight differences were not statistically significant presumably due to the short nature of the study (7 weeks duration).

Conclusion

This study showed that there was a slight but elevation of blood pressure ($p = 0.3783$) when rabbits are given 1.2% saline drink in place of water for a short duration of time (7 weeks). This suggests that unlike rats that became hypertensive on administering 1.2% saline drink for a short duration of 4 weeks (Dahl, 1962; Miyajima & Bunag, 1985; Obiefuna *et al*., 1991; Sofola *et al*., 2002), a longer period may be required to achieve hypertension by saline drink administration in rabbits. Also, further work is required on rabbits to know the exact saline concentration to be administered and the duration for its administration to be able to achieve elevation of blood pressure for cardiovascular studies.

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