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Amlodipine Corrects Changes in Blood Pressure and Baroreceptor Reflex Sensitivity in Sprague Dawley Rats Fed a High Salt Diet

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Summary: High dietary salt ingestion causes elevated blood pressure both in humans and in experimental animals. Following reports that Calcium Channel Blockers may exhibit differences in the response of blood vessels to pressor agents, this study sought to test the effects of amlodipine on blood pressure (BP) and baroreceptor reflex sensitivity (BRS) responses to high salt ingestion. Three groups of weanling Sprague-Dawley rats i.e. control rats (CR), salt loaded rats (SR) and salt loaded rats concomitantly administered orally with amlodipine (SR+Am) were used. At the end of 6-week experimental period, terminal arterial BP was determined from one femoral artery. BRS was calculated from the change in heart rate per change in mean arterial BP following bilateral carotid artery occlusion. Plasma sodium and potassium ion concentrations were also determined. Results show that dietary salt loading in SR significantly increased systolic and diastolic BP and Na^+ concentration but decreased BRS and K^+ concentrations significantly. These changes were abolished in the (SR+Am) rats indicating the ability of amlodipine to ameliorate the increase in blood pressure, reduction in baroreceptor reflex sensitivity and alterations in plasma Na^+ and K^+ levels that were observed in SD rats fed a high salt diet.

Keywords*:* Salt, Amlodipine, Baroreflex sensitivity, Blood pressure

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INTRODUCTION

Hypertension or high blood pressure is the biggest cause of morbidity and mortality in humans (World Health Organization, 2013). The condition has also been reported to be compounded by increase in salt intake in the diet (Meneton *et al*., 2005, Elias *et al.*, 2011). The large scale INTERSALT study had earlier reported correlation between salt intake, as estimated by the 24hour excretion of sodium, and level of arterial blood pressure in an international study on 10,500 subjects in several countries (Intersalt, 1988). The observation of elevated blood pressure in response to high salt intake has been aptly demonstrated in experimental animals such as such as rats e.g. Dahl Salt Sensitive (DS) rats, Spontaneously Hypertensive or SH rats, Sprague Dawley rats (Miyajima and Bunag, 1985; Obiefuna *et al.*, 1991; Sofola *et al.*, 2002), dogs (Vogel, 1966; Hainsworth *et al.*, 2003) and chimpanzees (Denton et al., 1995). Dietary salt loading with 8% sodium chloride in weanling SD rats consistently results in hypertension in these rats (Miyajima and Bunag, 1985, Sofola *et al.*, 2002). This was first demonstrated by Dahl in the development of the genetic Dahl Salt Sensitive rats (Dahl *et al.*, 1962). Some of the mechanisms reported for this elevated blood pressure in response to salt load include enhanced constrictor response of vascular smooth muscle to agonists as well as reduced vasodilatory responses in perfused rat mesenteric artery (Sofola *et al.*, 2002) and in pressurized muscle resistance arteries (Weber and Lombard, 2000).

The elevated blood pressure response to high dietary salt intake in rats has been shown to be ameliorated by some agents such as ACE inhibitors like captopril (Wyss *et al*., 1994) as well as the aldosterone antagonist, spironolactone (Sofola and Adegunloye, 1998), implicating the Renin Angiotensin Aldosterone System (RAAS) in the response. Calcium ion Channel Blockers (CCBs) have been used extensively to treat human hypertension either as monotherapy or in combination therapy, especially in blacks (Flack and Robert, 2009). These compounds including the dihydropyridines (eg nifedipine and amlodipine) and non-dihydropyridine compounds (eg diltiazem and verapramil) lower blood pressure by reducing the entry of calcium ions into the heart and blood vessels (Frishman, 2007). However, an earlier report showed that the calcium channel antagonist, nifedipine, does not abolish the high blood pressure response to salt loading in SD rats (Nwaigwe and Sofola, 1989). In view of the reported differences in the vascular actions of different CCBs (Xu *et al*., 2002), it is possible that

these agents may not be acting solely as vascular calcium ion channel blockers, in reducing blood pressure and that there may be other additional mechanisms involved.

The present experiments were therefore designed to test the effects of another CCB, amlodipine, on the high blood pressure response to ingestion of high dietary salt intake in Sprague Dawley rats, by determining its effects on the level of arterial blood pressure, baroreceptor reflex sensitivity as well as plasma concentrations of sodium and potassium ions.

MATERIALS AND METHODS

Experiments were carried out on twenty-eight weanling 6-weeks old Sprague-Dawley rats weighing between 110-120 grams. The animals were divided into three experimental groups. Control Rats (CR, n=10) given normal rat chow and water ad libitum. The second group or Salt loaded rats (SR, n= 10) were rats that were fed on rat chow containing 8% sodium chloride as described earlier (Sofola et al., 2002); while the third group were Salt-loaded rats that were concomitantly administered with amlodipine (SR+Am, n=8). The amlodipine was dissolved in water and given by oral gavage at a dose of 0.3mg/kg body weight. This dose corresponds to a therapeutic dose of 20mg in an average 70kg man. These procedures were carried out for 6 weeks. At the end of the experimental period, the rats were anaesthetized with a solution of 25% (w/v) urethane and 1% (w/v) α-chloralose injected intraperitoneally at a dose of 5 ml/kg body weight. The anaesthetized rat was placed on dissecting board and the trachea was cannulated. The blood pressure measurements were obtained by cannulation of one femoral artery. A polyethylene cannula filled with 1% heparinised saline was inserted into the artery, tied in place, and connected via a pressure transducer (model SP 844, Physiological Pressure Transducer. AD Instruments) that was attached through MLAC11 Grass adapter cable to a computerized data acquisition system with LabChart-7 pro software (Power Lab-4/24T, model MLT844/P; AD Instruments Pty Ltd., Castle Hill, Australia). The heart rate was determined by counting the number of arterial pulses over a period of 60 seconds. Both common carotid arteries were isolated in the neck and sutures put around them for subsequent bilateral carotid occlusion.

In 5 rats from each group, the blood pressure and heart rate response to bilateral carotid occlusion (BCO) were determined by applying bulldog clips to both common carotid arteries. BCO was carried out twice in each rat at 10 minutes interval with each occlusion lasting for 20 seconds. Baroreflex sensitivity was calculated as change in heart rate per unit change in mean peak arterial blood pressure (measured in beats.min^{-1}.mmHg^{-1}) after bilateral carotid artery occlusion. At the end of all experiments, blood was obtained by cardiac puncture and transferred into lithium heparinised tubes which were then centrifuged to obtain plasma sample for determination of Na^+ and K^+ concentrations in the plasma.

Statistical analysis.

Data were expressed as Mean ± SEM. The values were analyzed using Student's t-test and one-way Analysis of Variance (ANOVA). Values were considered statistically significant $p < 0.05$.

RESULTS

Terminal Blood Pressure and Heart Rate

The systolic blood pressures (SBP) in the three groups of rats were CR 104.5±5.6 mmHg, SR 150.7±2.5 mmHg and SR+Am 93.6±8.0mmHg respectively. SBP in SR was significantly ($p<0.001$) higher when compared with that of CR. It was however lower significantly ($p<0.01$) in SR+Am compared to the SR group. For diastolic blood pressure (DBP), the corresponding values were 78.8±4.3mmHg, 118.9±3.4 mmHg and 70.9±5.4 mmHg for CR, SR and SR+Am respectively. DBP was significantly higher in SR than in CR ($p<0.001$) but significantly lower in SR+Am than in SR ($p<0.001$). Mean Arterial Blood Pressure (MABP) was significantly higher ($p<0.001$) in SR 129.5±2.9 mmHg compared with CR 87.3±4.6mmHg but significantly ($p<0.05$) lower in SR+Am 78.5±6.0 mmHg when compared with SR ($p<0.001$). These results are summarized in Table 1 and Figures 1 and 2 for SBP and DBP respectively. Heart rate responses were not significantly ($p>0.05$) different between CR and SR. However, the rate was reduced but not significantly in the SR+Am group compared to the SR group.

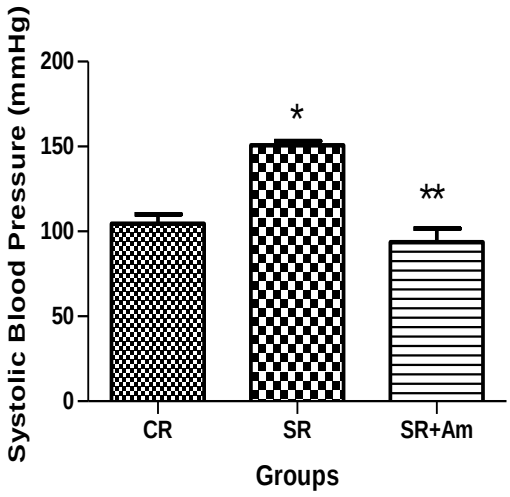

Figure 1. Terminal systolic blood pressure in the different groups of rats. CR= Control Rat, SR= Salt-loaded Rat, SR+Am=Salt-loaded and administered with amlodipine. *=Significant ($p<0.001$) compared with CR, **=significant decrease ($p<0.001$) compared with SR.

Table 1: Blood Pressure Parameters and Heart Rate in all groups.

Groups	SBP (mmHg)	DBP (mmHg)	MABP(mmHg)	HR (beats/min.)
Control (CR) (n=10)	104.5±5.6	78.8±4.3	87.3±4.6	355.1±4.0
Salt-loaded (SR) (n=10)	150.7±2.5*	118.9±3.4*	129.5±2.9*	353.6±2.6
Salt + Amlodipine (SR+Am) (n=8)	93.6±8.0**	70.9±5.4**	78.5±6.0**	329.9±2.3

Values represent Means ± SEM. *Significant increase ($p < 0.001$) when compared with CR. **=Significant decrease ($p < 0.001$) when compared with SR

Table 2: Baroreflex Sensitivity and Plasma Na^+ and K^+ Concentrations in all groups

Groups	BRS (beats $min^{-1}mmHg^{-1}$)	Na^+ (mmol/L)	K^+ (mmol/L)
Control (CR) (n=10)	0.56±0.01	136.70±1.17	5.12±0.04
Salt-loaded (SR) (n=10)	0.42±0.05*	144.70±2.41 β	3.87±0.11*
Salt + Amlodipine (SR+Am) (n=8)	0.54±0.02 μ	133.5±2.54**	5.02±0.07 μ

Data are presented as means ± SEM. *Significant decrease ($p < 0.05$) when compared with CR. **Significant decrease ($p<0.01$) when compared with SR. β=Significant increase ($p < 0.05$) when compared with CR. μ =Significant increase ($p < 0.01$) when compared with SR

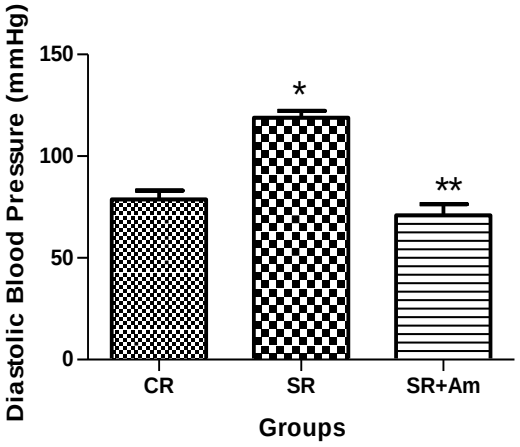

Figure 2. Terminal diastolic blood pressure in the different groups of rats. CR= Control Rat, SR= Salt-loaded Rat, SR+Am=Salt-loaded and administered with amlodipine. *=Significant ($p<0.001$) compared with CR, **=significant decrease ($p<0.001$) compared with SR group.

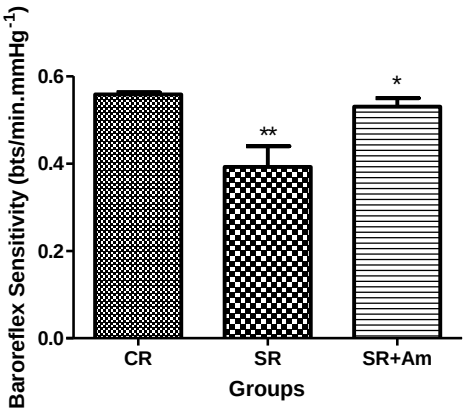

Figure 3: Calculated baroreflex sensitivities in the different groups of rats. CR= Control Rat, SR= Salt-loaded Rat, SR-Am=Salt-loaded and administered with amlodipine. **Significant decrease ($p < 0.05$) when compared with CR. * = Significant increase ($p <0.01$) when compared with SR.

Baroreflex Sensitivity

Baroreflex sensitivity ($beats.min^{-1}.mmHg^{-1}$) as determined for each groups were 0.57±0.01, 0.42±0.08 and 0.54±0.13 in the CR, SR and SR-Am respectively. The value was significantly ($p<0.05$) lower in the SR ($p<0.05$) compared to the CR. The value was however significantly higher ($p<0.01$) in the SR+Am group when compared with the SR group. The values for CR and SR-Am were however not significantly different. This is shown in Table 2 and Figure 3.

Plasma Na^+ and K^+ Concentration

Plasma Na^+ concentration was significantly higher ($p<0.05$) in the SR group when compared with the CR group. In the SR+Am group however, the plasma Na^+ concentration was significantly lower ($p<0.01$) than in the SR group. On the other hand, K^+ concentration was significantly lower ($p<0.05$) in the SR group compared to the CR group while it was significantly higher ($p<0.01$) in the SR+Am group compared to the SR group. Plasma sodium and potassium concentrations in CR and SR-Am were not significantly different.

DISCUSSION

The results of the present experiments have confirmed that in weanling SD rat's ingestion of a high salt diet resulted in hypertension, in confirmation of earlier reports (Miyajima and Bunang, 1985; Sofola *et al.,* 2002; Oloyo *et al*., 2011). The high blood pressure in response to dietary salt loading has been ascribed to many factors which include the responsiveness of vascular smooth muscle (VSM) as it has been reported that salt loading results in enhanced vasoconstrictor responses to agonists especially norepinephrine (Sofola *et al.,* 2002) as well as reduction in vasorelaxation response to vasodilators such as acetylcholine (Weber and Lombard, 2000; Sofola *et al.,* 2002). These vascular muscle responses may be additive and hence result in an increase in blood pressure. However, in addition to the VSM responses,

additional mechanisms may include the changes in serum concentrations of K^+ and Na^+ ions, observed in the present experiments, similar to some earlier observations (Obiefuna *et al.,* 1991). Some reports have shown that low plasma K^+ concentration may have an inverse correlation with arterial blood pressure (Pikilidou *et al.,* 2007) while K^+ supplementation in salt fed rats reduced the elevated blood pressure as well as impair vasoconstrictor responses to noradrenaline (Sofola and Adegunloye, 1998). Similarly, in humans, K^+ rich diet such as the DASH diet reduces level of arterial blood pressure (Sacks *et al*., 2001). Additionally, elevation of serum Na^+ is a major factor in increasing blood pressure (Adrogué and Madias, 2007).

In the present experiments, oral amlodipine administration reversed the high blood pressure response to a high salt diet in the SD rats. This observation was accompanied by the return of K^+ and Na^+ concentrations to similar levels to those in normal/control rats. Thus, though amlodipine is presumed to act majorly as an L-type calcium ion channel antagonist in the heart and vasculature, it thus appears that its actions in correcting both the hypokalemia and the hypernatremia are important additional factors in the lowering of the arterial blood pressure in salt-induced hypertension. Some other factors that have been implicated in the mechanisms of action of amlodipine also include its ability to cause the release of endothelial vascular Nitric Oxide (Xu *et al*., 2002, He *et al*., 2014) as well as inhibition of angiotensin converting enzyme (ACE) (Xu et al., 2002) and these eNOS and ACE-dependent effects of amlodipine were reported to be negligible in the vascular responses to verapamil and nifedipine (Xu *et al*., 2002). These observations may therefore explain why in an earlier study, we did not observe a reduction of the elevated blood pressure in salt loaded rats that were administered with nifedipine (Nwaigwe and Sofola, 1989) in contrast to the present observations with amlodipine.

Amlodipine administration in this present experiment reversed the reduction in baroreceptor reflex sensitivity (BRS) in response to bilateral carotid occlusion in the salt loaded rats. Blunting of BRS in Salt loaded rats had earlier been reported (Miyajima and Bunag, 1985). It thus appears that there may have been the resetting of BRS during salt loading and this reduction was corrected by amlodipine. There is now heightened interest in the use of baroreceptor stimulation as effective control of hypertension (Bisognano *et al.,* 2011). Also, it has been reported that reduction in the raised blood pressure in renovascular hypertension increases baroreflex sensitivity (Queiroz *et al.,* 2014). Thus, the action of amlodipine in correcting the depressed BRS induced by high salt diet, will be additional mechanism in lowering the high blood pressure that was observed in the salt loaded rats.

In conclusion, the results of our present experiments indicate that administration of amlodipine during salt loading in SD rats will blunt the pressor response of salt through mechanisms that include correction of changes in serum Na^+ and K^+ concentrations, as well as correction of depressed baroreceptor reflex sensitivity.

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