Research Article

Chronic exposure to high environmental temperature exacerbates sodium retention and worsens the severity of salt-induced hypertension in experimental rats via angiotensin receptor activation

F.M. Agbaraolorunpo*, A.K. Oloyo, C.N. Anigbogu and O.A. Sofola

[1]*Department of Physiology, College of Medicine of the University of Lagos, Lagos, Nigeria; Department of Physiology, College of Medicine of the University of Lagos, Lagos, Nigeria.*

Keywords:

Angiotensin II receptor; high salt diet; hot environment; environmental temperature; heat exposure; hypertension

ABSTRACT

Background: There is paucity of information on how exposure to high environmental temperature interacts with high dietary salt to influence cardiovascular outcome in the face of global warming. **Method:** This study investigated the impact of high environmental temperature (HET) on the cardio- renal indices of an animal model of hypertension (fed on high salt diet, HSD), and evaluated the effectiveness of angiotensin II receptor blocker (ARB), telmisartan, in modulating these indices. Fifty-six male Sprague Dawley rats (70-90g , 7 week old) were randomly assigned into seven groups of 8 rats, which include control rats (I), fed 0.3% NaCl diet; salt loaded rats (II), fed with 8% NaCl (high salt) diet; Heat rats (III), exposed to HET (37.5-38.5°C) 4 hours daily per week; salt loaded + Heat rats (IV), fed with 8% NaCl diet and exposed to HET daily. Next, salt loaded + ARB rats (V) , fed 8% NaCl diet and treated with telmisartan (30mg/kg); Heat +ARB rats, exposed to HET and treated with telmisartan (30mg/kg); salt loaded +HET+ARB rats (VI), fed with 8% NaCl diet, exposed to HET and treated with telmisartan (30mg/kg). Experiment lasted 8 weeks. Blood Pressure and heart rate were determined invasively and electrolytes by selective ion electrode method. Data analyzed using ANOVA, with $P<0.05$ significant. **Results:** Systolic and Diastolic BP, Mean Arterial Pressure, Rate Pressures Product (RPP) ($P<0.05$), and plasma Na$^+$ ($P<0.05$) were significantly higher with associated suppressed Na$^+$ excretion ($P<0.05$) in salt loaded rats exposed to HET compared to rats fed a high salt diet alone. Telmisartan significantly attenuated the elevated blood ($P<0.05$) and RPP ($P<0.05$), in the HSD rats exposed to HET, with no corresponding reduction in the rats fed a HSD alone. **Conclusion:** This indicates that chronic exposure to hot environment exaggerated cardiovascular response to high salt diet possibly via angiotensin II pathway with consequent enhanced ARB action.

INTRODUCTION

Persistent blood pressure elevation, otherwise referred to as Hypertension, is common and more severe among blacks than whites (van de Vijver et al., 2013) . It is reported as the principal cause of cardiovascular diseases (CVDs), which has been identified as the leading cause of global death (WHO, 2017). Specifically, African region accounted for the highest prevalence of hypertension put at 46% of the reported 1billion cases in 2008 (Cappuccio and Miller, 2016).

*Address for correspondence:
Email: fagbaraolorunpo@unilag.edu.ng
Tel: +234 8026441896

High salt diet is a major risk factor (Mozaffarian et al., 2014) in the development of hypertension especially among the black population (Svetkey et al., 1999) . This form of hypertension, referred to as salt-induced hypertension, is characterized by increased tendency of sodium ion retention by the kidney. This is critical as the kidney is involved in the regulation of body water and sodium balance which is key to long-term blood pressure regulation (Meneton et al., 2005).

Historically, salt retention by the kidney was a survival strategy against dehydration by the black man transported in hot environment during the slave era, as postulated by the controversial Wilson-Grim slavery hypertension hypothesis (Wilson and Grim, 1991) and in

agreement with Curtin's historical perspective (Curtin, 1992). Unfortunately, salt consumption in diets is increasingly becoming high, with sodium level averaging between 2.18 and 5.51g in 181 of 187 countries around the world (Bernstein, 2014) and in many African countries (Cappuccio et al., 2006).This is well above the recommended reference level of 2.0g of sodium intake per day (Mozaffarian et al., 2014).

Meanwhile, as a result of global warming caused by the indiscriminate emission of green gas into the atmosphere (MacMillan , 2016), high environmental temperature (HET) has been suggested as the number one weather-related killer, causing more fatalities per year than flood, lightening, tornadoes, hurricanes, winter storms and extreme cold (National Oceanic and Atmospheric Administration, 2012). Therefore, climate change is considered to be a major challenge of the 21th century according to experts (Hajat et al., 2014). For example, on hot days, temperatures inside parked automobiles can be as high as 57°C–78°C (McLaren et al., 2005). In support of this narrative, high environmental temperature has been implicated as a risk factor in the development of cardiovascular diseases (CVDs) (Moghadamnia et al., 2017). Sadly, sub-Saharan African region is a tropical region characterized by high climate temperature with associated high prevalence of occupational heat exposure (Collins, 2011).

Hitherto, there is paucity of information available on the possible interaction between high salt diet and long term exposure to environmental heat on the cardiovascular function. It is not also known if the rising global temperature and the adverse workplace environmental temperature of low income countries, particularly in sub-Saharan African countries, play any role in the pathophysiology of the severity of salt-induced hypertension in this global subset. Both factors are known to modulate sympathetic nervous system and Renin Angiotensin System (RAS).

Meanwhile, it has been suggested that the use of Angiotensin receptor blocker (ARB) as a monotherapy in the control of hypertension may not be effective in black populations compared to whites (Mancia et al., 2007) . However, a 6- week clinical trial with telmisartan, an ARB, on hypertensive black patients suggested otherwise (Mabayoje & Oke,2004). The mechanisms by which ARB(s) control blood pressure among the black is largely unknown. But evidence suggest that the mechanism of action of these agents could be influenced by a phenomenon referred to as salt sensitivity (Endo et al., 2009) . This is a phenomenon characterized by the tendency of blood pressure to rise with high level of salt diets (Weinberger et al., 1986). We therefore hypothesized that long-term exposure to hot environmental temperature worsens cardiovascular adaptive adjustment to high salt diet. The basis of our hypothesis is that, environmental heat may modulate the therapeutic potential of ARB in salt-induced hypertension.

METHODS
Animals
 Fifty-six male Sprague- Dawley rats were used for the study. The rats were obtained from the animal house of the College of Medicine of the University and were housed in standard plastic cages at the animal unit of Physiology Department of College of Medicine, University of Lagos, Nigeria. The rats were maintained on a 12h dark/light cycle at 26-29°C ambient temperature in the animal house. They were allowed access to standard rat chow and clean tap water ad libitum throughout the study and were allowed to acclimatize for 1 week before the experimental procedures in standard cages at the animal house of Department of Physiology. Animal care and handling was done according to the National Research Council (US) Committee for the Care and Use of Laboratory Animals, 2011). The study protocol was approved by the Ethics committee of College of Medicine of the University of Lagos (CMUL/HREC/11/18/471).

 Experimental Design
The rats were randomly assigned into 7 groups of 8 rats per group as shown in Table 1.They were either fed on normal salt diet (0.3%NaCl) or high salt diet (8%NaCl); exposed to ambient room temperature (26-29°C) or high environmental temperature (37.5-38.5±0.5°C 4hr daily per week for 8 weeks); untreated or treated with oral telmisartan (30mg/kg daily for 7 weeks).

Experimental procedure:
Salt loaded rats (Groups II, IV, V and VII) were fed on high salt diet as described by (Sofola et al., 2002) for 8 weeks. The high salt diet contained 7.7% NaCl added to the original diet containing 0.3% NaCl.

Exposure to hot environment/ high environmental temperature (HET)
Prior to the exposure of animals to HET, the animals were acclimatized to HET for one week at a temperature between 30 and 35°C. Thereafter, the animals were exposed to HET at 37.5-38.5±0.5°C using the method described by Barney and Kuhrt (2016) with some slight modification. Briefly, exposure took place for 4 hours daily for 6 days per week in 8 weeks, in an environmental chamber from 9am to 1pm. The environmental temperature was monitored with an

Table 1: Allocation of animals into experimental groups

Groups (n=8)		Feeding	Experimental Temperature	Treatment
		8 weeks	8 weeks	7weeks
I	Control	Normal rat chow (0.3% NaCl)	Room temperature	Nil
II	Salt loaded	High salt diet (8% NaCl)	Room temperature	Nil
III	Heat exposed	Normal rat chow (0.3% NaCl)	High ambient temperature(37.5-38.5±0.5°C)	Nil
IV	Salt + Heat	High salt diet (8% NaCl)	High ambient temperature (37.5-38.5±0.5°C)	Nil
V	Salt+ARB	High salt diet (8% NaCl)	Room temperature	Telmisartan (30mg/kg)
VI	Heat+ARB	Normal rat chow (0.3% NaCl	High ambient temperature (37.5-38.5±0.5°C)	Telmisartan (30mg/kg)
VII	Salt+Heat+ARB	High salt diet (8% NaCl)	High ambient temperature(37.5-38.5±0.5°C)	Telmisartan (30mg/kg)

environmental digital LCD thermometer (HTC-2 model). Core temperature was determined before and after exposure to HET by gently inserting the sensor of a digital thermometer inside the animal anus (Omron, MC246). The assessment was done in the first and last week of the exposure. The environmental chamber was made of a plastic cage (30cm by 50cm by 23 cm) with wide aluminium net for good ventilation. The source of HET was a 100Watt dry bulb positioned on the roof of the cage to generate heat that raises the temperature in the chamber. The chamber had wooden shaves for bedding. Feed and water were not made available to rats until the exposure was over.

Administration of Drugs to Animals
The angiotensin II receptor blocker (ARB) treated rats had 30mg/kg per body weight oral doses of telmisartan (Gohlke et al., 2001) administered to them daily with oral cannula. The treatment commenced a week after introducing the animals to high salt diet and HET respectively. The drug was obtained from MSN Laboratories Private Limited, India through Phillip Pharmaceutical Nigeria Limited.

Determination of Sodium (Na^+) clearance, Na+ excretion and Glomerular Filtration Rate
12-hour urine output (V) in ml was determined at 8th week of the experiment from 7 p.m to 7 a.m in a metabolic cage. Urinary Na^+ excretion was evaluated as product of urinary Na^+ concentration (U_{Na}^+) and 12hr-urine volume(V) in (mmol/L/12hr); Plasma Na^+ clearance was calculated as urinary Na^+ excretion (U_{Na} V) divided by Plasma Na^+ concentration (P_{Na}^+) in (ml/min).This is also bearing in mind the validation of 12-hr urine collection for the calculation of sodium excretion as an index of dietary sodium intake(Mill et al., 2012) . While GFR was estimated as a product of

urinary creatinine concentration and urine volume, divided by plasma creatinine concentration, $U_{cr}V$ (min) /P_{cr} (ml/min). Plasma and Urine electrolytes (Na^+, K^+, Cl^-) in mmol/L were determined with ion selective electrode method (ISE 6000 analyzer, France); Plasma and urine Creatinine concentration were determined with alkaline picrate colorimetric method followed by analysis with Spectrophotometer (SFRI, BSA-3000 Chemistry analyzer, France).

Animal Anaesthesia
The rats were sacrificed at the end of week 8 of the experiment. Solution of 25%(w/v) urethane and 1% alpha chloralose were administered as anaesthesia via intraperitoneal route (i.p), at a dose of 5ml/kg body weight(Oloyo et al., 2019). Blood was collected from the rat heart via cardia puncture and placed in heparin bottles for electrolytes and creatinine determination and stored at-20°C following the measurement of blood pressure and heart rate. Plasma was separated from blood after 15 minutes of centrifugation at 3000 rev per min.

Blood pressure and Heart Rates Measurement
Blood Pressure and Heart Rate (HR) were determined via cannulation of carotid artery (Oloyo et al., 2011) with polyethylene cannula, connected to 1% heparinized normal saline with a 3-way channel connector. The cannulated artery was secured tightly with thread and connected to Power lab pressure transducer (model SP844, Physiological Pressure Transducer, AD Instrument (Power Lab-4/24T) which was in turn attached through MLACH11 Grass adapter cable to a computerized data acquisition system with Labscribe software. Heart rates were determined by counting the number of arterial pulses at sampling frequency of sample of 5/s. The Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) were obtained directly

from the tracing as the peak and the base of the arterial pulses respectively. Mean Arterial Pressure (MAP) was determined from the following formula:1/3(SBP-DBP) + DBP. Rate Pressure Product (RPP), a representative of myocardial workload and a predictor of myocardial oxygen consumption(MOD), was determined as a product of SBP and HR (Gobel et al., 1978) .

Statistical Analysis
Data are presented as Mean ± SEM. Differences in experimental rat groups were compared with one- way ANOVA followed by Tukey post-hoc test, Pre and post rectal temperatures were compared with t-test. Graph pad 5 software package was used for the analysis. Statistical significance set at P<0.05.

RESULTS
Effect of chronic exposure to hot environment on the behaviour of the Sprague- Dawley rats
Rats exposed to high environmental temperature were observed to develop rough and erect furs, with dampen lower body surface and furs, especially in the salt plus heat group. Furthermore, the rats licked each other tongues, hid themselves under bedding in the environmental chamber, away from heat source, breath faster and deeper and the animal looked smaller. Occasionally, the rats were hyperactive and standing erect. Excessive jumping indicated imminent heat shock, and exposure was discontinued at this stage. Two animals developed heat stroke in the heat+ ARB at 5th and 7th week of exposure with signs of spinning, falling on one side, following growth retardation and died subsequently. Two rats also developed heat shock in the salt+heat group characterized with sudden death during the early week of heat exposure.

Effect of chronic exposure to hot environment on rectal temperature in salt loaded rats treated with telmisartan
Rectal temperatures, an index of core temperature, was significantly raised (P<0.001) by exposure to high environmental temperature in all the rats exposed to high environmental temperature. The mean core temperature for the unexposed animals (n=23) was 35.5±0.2°C which was not significantly (P=0.08) different from the pre-exposure core temperature of 36.0±0.1°C for the heat exposed rats (n=32).

Effect of chronic exposure to hot environment on blood pressure, heart rate and Rate pressure product salt loaded rats treated with telmisartan
In this study (figure 1 & table 2), the feeding of Sprague - Dawley rats with high salt diet (8% of NaCl) for 8 weeks resulted in significantly higher SBP (P<0.01), DBP (P<0.01), HR(P<0.05) and RPP (P<0.01) compared to control. Similar higher records were observed for SBP (P<0.05), DBP (P<0.05), MAP

Table 2: Effect of high environmental temperature on core temperature of Sprague-Dawleys rats

Groups	Core Temperature		
	Pre-exposure	Post-exposure	Temperature Δ
Heat	35.7±0.2	37.8±0.1***	2.1±0.4
Salt+heat	36.0±0.2	38.6±0.4***	2.6±1.3
Heat+ARB	36.4±0.2	39.0 ± 0.3***	2.6±1.0
Salt+Heat+ARB	36.3±0.1	38.1±0.2***	1.8±0.8

Exposure of Sprague-Dawley rats to hot environment of 37.5-38.5±0.5°C ,4 hours daily, 6 days per week in 8 weeks significantly raised the rectal temperatures of the rats. Data presented as Mean±SEM ;t-test and One-way ANOVA with Tukey post test. (***P<0.001 vs pre exposure).

(P<0.05), HR (P<0.05) and RPP (P<0.05) in rats chronically exposed to hot environment when compared to control. However, a combination of high salt diet and exposure to hot environment in the salt + heat group resulted in a significantly much higher elevation of SBP (P<0.001), DBP (P<0.001), MAP(P<0.001), and RPP(P<0.001) relative to control, and in comparison with rats fed a high salt diet: SBP (P<0.01), DBP(P<0.01), MAP(P<0.01), RPP(P<0.01) and compared to rats exposed to hot environment alone: SBP(P<0.001), DBP (P<0.001), MAP(P<0.001), RPP(P<0.001). These results suggest that long term exposure to high environmental temperature increased blood pressure, HR and myocardial work load in the studied rats comparable to high salt diets feeding, while a combination of the two factors resulted in an additive effect on blood pressure and myocardial workload.

Meanwhile, temisartan significantly attenuated the elevated SBP (P<0.001) DBP (P<0.001) MAP (P<0.001) and RPP (P<0.001) in rats exposed to hot environment plus fed a high salt diet in comparison with the untreated rats exposed to the same combined environmental conditions. Similarly, treatment with telmisartan significantly lowered SBP (P<0.05) and RPP (P<0.05) in rats exposed to hot environment alone in comparison with untreated rats under the same condition. Conversely, telmisartan treatment produced no noticeable reduction in SBP (P>0.05), DBP (P> 0.05), MAP (P>0.05) and RPP (P>0.05) in rats fed a high salt diet alone. Put together, blockade of AT1 receptor with telmisartan resulted in significant attenuation of the elevated SBP, DBP, MAP, and myocardial workload in salt loaded rats exposed to HET, with no reduction in rats fed with high salt diet alone. Similar telmisartan-mediated attenuations were recorded in the SBP (P<0.05) and RPP (P<0.05) of rats exposed to hot environment alone.

Table 3: Effect of hot environment on blood pressures and heart rate in the presence and absence of high salt diet in male Sprague-Dawley rats

Groups	Rats	Systolic BP mm Hg	Diastolic BP mm Hg	Mean Arterial BP mm Hg	Heart Rate Bpm
I	Control (n=8)	110.0±4.7	77±2.0	87.6±2.4	330±15
II	Salt (n=8)	137.0±4.7**##	108±5.0**##	118.4±4.2**#	396 ±11**
III	Heat (n=8)	133.0±5.0*###	100±9.0*###	111±7.3*###	399±10**
IV	Salt+ heat (n=6)	168.0±7.2***	141±5.0***	149.9±5.5***	420±90***
V	Salt+ARB (n=8)	149±5.9	123±4.0	131.7±4.6	408±16
VI	Heat+ARB (n=6)	105.0±5.9††	85.±6.0	91.7±5.9	384±15
VII	Salt+heat+ARB(n=7)	114.0±5.1†††	88±6.0††	96.70±5.6†††	407±11**

Rats fed with normal salt (0.3%NaCl) or high salt diet (8%NaCl) were either exposed to high environmental temperature of 37.5-38.5±0.5ºC ,4 hours daily, 6 days per week for 8 weeks or maintained on room temperature (26-29±0.5ºC) with or without telmisartan (30mg/kg body weight) treatment. Data presented as Mean±SEM. One-way ANOVA with Tukey post test;*P<0.05, **P<0.01, ***P<0.001 is significantly higher than control; ##P<0.01, ###P<0.001,†††P<0.001 is significantly lower than Salt+ heat (IV);††P<0.01 vs is significantly lower compared to Heat (III).

Table 4: Effect of hot environment on plasma electrolytes, urea and creatinine in the presence and absence of high salt diet in male Sprague-Dawley rats.

Groups	Na⁺ (mmol/L)	K⁺ (mmol/L)	Cl⁻ (mmol/L)	Creatinine (Cr, mg/dl)	Urea mg/dl
control	134.3±0.8	4.5 ± 0.2	105.3 ± 0.8	1.2 ± 0.15	15.3±0.9
salt	141.1±1.2*	6.1± 0.4	104.1 ± 2.3	1.42 ± 0.17	24.0±2.2
heat	141 ± 1.8*	6.6 ± 0.7	101.0 ± 2.7	1.19 ± 0.19	23.3±2.4
Salt + heat	148.3±0.7***#	5.0 ± 0.5	106.8 ± 1.3	1.18 ± 0.14	25.1±1.6
Salt+ARB	140.8±2.7	6.1 ± 0.5	105.4 ± 1.7	1.84 ± 0.20	19.8±1.9
Heat+ARB	144.6±1.3**	4.7 ± 0.4	102.3 ± 1.1	1.28 ± 0.16	35.4±5.0
Salt+Heat+ARB	143.5±1.0**	5.3 ± 0.5	105.3 ± 1.7	0.81 ±0.09	18.4±0.9

Effect of 8 weeks of exposure to high environmental temperature on Plasma electrolytes in rats fed a high salt (8%NaCl) or normal salt diet (0.3%NaCl) with or without treatment with Telmisartan (30mg/kg/ bw orally). *P<0.05, **P<0.01 ***P<0.001 is significantly higher compared to control; #P<0.05 is significantly higher compared to salt.

Effect of chronic exposure to hot environment on Plasma Na⁺, K⁺, Cl⁻ in salt loaded rats treated with telmisartan
Table 3 shows that in comparison with control rats, plasma Na⁺ was significantly higher in rats fed with high salt diet alone (Na⁺=134.3±0.8 vs 141.1±1.2mmol/L; P<0.05) and in rats exposed to hot environment alone (Na⁺=134.3±0.8 vs 141 ± 1.8mmol/L; P<0.05), as well as in rats fed a high salt diet plus exposed to hot environment (Na+=134.3±0.8 vs 148.3±0.7mmol/L; P<0.001). But plasma Na⁺ level in rats fed a high salt diet plus exposed to hot environment was significantly higher compared to rats fed with high salt diet alone

(148.3±0.7 vs141±1.8mmol/L, P<0.05). This result suggests that interaction between high environmental temperature and high salt diet, apparently resulted in additive elevation of plasma Na⁺.

Meanwhile, telmisartan lowered plasma Na⁺ level in rats fed a high salt diet plus exposed to hot environment noticeably compared to the untreated rats under same condition (143.5±1.0 vs 148.3±0.7mmol/; P>0.05). However, this did not reach significant level (P>0.05) Conversely, plasma Na+ level increased slightly in telmisartan- treated rats exposed to hot environment alone (144.6±1.3 vs 141±1.8mmol/L) compared to the

Table 5: Effect of 8 weeks of exposure to HET on $U_{Na}V$, Na+ clearance & GFR in rats fed a high salt (8%NaCl) or normal salt diet with or without treatment with Telmisartan (30mg/kg/ bw orally)

Groups	$U_{Na}V$ (mmol/L/12hr)	Na$^+$ clearance (μmol/ml/min)	GFR(ml/min)
control	462.3±130.1	5.1±0.9	0.0051±0.02
salt	1292±254.5 **	12.8±2.6*	0.012±0.04
heat	547.5± 119.9[#]	5.4±1.2[#]	0.0054±0.03
Salt+heat	437.5±76.27 [##]	4.1±0.7[##]	0.0041±0.05
Salt+ARB	949.1±133.3	10.2±2.0	0.0102±0.01
heat+ARB	284.2±23.21	2.7±0.2	0.0027±0.02
Salt+heat+ARB	569.8±88.38	5.5±0.8	0.0.55±0.05

.*P<0.05 & **P<0.01 higher than control; [#]P <0.05 and [##]P<0.01 lower than Salt group .Data presented as Mean±SEM and One-way ANOVA with Tukey post test

untreated rats exposed to hot environment alone; whereas the level was unaffected in telmisartan- treated salt-loaded rats in comparison (140.8±2.7vs 141.1±1.2mmol/L) to the untreated salt-loaded rats. This suggest that telmisartan did not significantly attenuate the aforementioned elevated plasma Na+ resulting from a combination of HET and high salt diet or the individual factors.   Furthermore, plasma K$^+$, Cl$^-$, creatinine and urea were not significantly affected by high salt diet, exposure to hot environment and telmisartan administration.

Effect of chronic exposure to hot environment on urinary sodium excretion, Na+ clearance and GFR in salt loaded rats treated with telmisartan
 In comparison with control (Table 4), rats fed a high salt diet had significantly higher urinary Na$^+$ excretion (P<0.01) and plasma Na+ clearance (P<0.05) , while rats exposed to hot environment (heat group) had slightly higher urinary Na$^+$ excretion with unchanged plasma Na$^+$ clearance.

Furthermore, rats fed a high salt diet and exposed to hot environment had slightly lower Na$^+$ excretion and Na+ clearance relative to control. As presented above, high environmental temperature in the presence of high salt diet resulted in significantly lower urinary Na+ and plasma Na+ clearance when compared with rats fed a high salt alone. This indicates that high environmental suppressed natriuresis in salt-loaded rats. However, GFR was unaltered in all the experimental rats.

DISCUSSION
    The finding of this current study reveals that rats fed a high salt diet and exposed to long term high environmental temperature (HET) developed a severe form of hypertension in comparison with rats exposed exclusively to hot environment or rats fed a high salt diet alone. Interestingly, the observed severity in the salt-induced hypertension in our animal models was ameliorated by angiotensin II receptor blockade with telmisartan treatment
    First, we observed that rats chronically exposed to hot environment had significantly higher Systolic (SBP) and Diastolic blood pressure (DBP), Mean arterial pressure (MAP) and Rate Pressure Product (RPP), an index of myocardial workload, when compared with control rats .This clearly supports   earlier studies by Geng et al.,(2016) and Hundekari (2012). Similarly, Brook et al., (2011) observed that BP was elevated in people following hotter night.
    Next, heart rate (HR) was noticed to increase significantly in the rats chronically exposed to hot environment compared to control. This was similar for the rats fed on high salt diet. This observation is in line with previous studies that acute and chronic heat exposure significantly raised HR (Alam et al., 2011 & Okoruwa,2014) . The heat-induced rise in HR arising from chronic exposure to high environmental temperature may be due to sympathetic nervous system activation as indicated by previous study(Yamamoto et al., 2007).
    Expectedly, our results provide further evidence in support of the involvement of high salt diet in the development of salt-induced hypertension, given the significant elevation of SBP, DBP, MAP, HR and RPP of rats fed a high salt diet compared to control, in agreement with previous studies (Elias et al., 2011; Malta et al., 2018). Since this form hypertension is preponderant among the black race (Lackland, 2014), we

reason that high environmental temperature in sub-Saharan Africa may play a key role in priming the kidneys of African descendants towards salt-induced hypertension, by suppressing the ability of kidney to effectively excrete salt, in tandem with previous historic and experimental evidences (Curtin, 1992; Lujan & DiCarlo, 2018).

In line with our hypothesis, the result of this current investigation clearly suggest that rats fed a high salt diet plus chronically exposed to hot environment, had significantly higher SBP, DBP, MAP and RPP relative to control and in comparison with rats exposed to hot environment alone as well as rats fed a high salt diet alone. These observations indicate that chronic exposure to high environmental temperature possibly amplified the potential negative effect of high salt diet on SBP, DBP and MAP and myocardial workload. The implication of this is that salt-related hypertension in salt sensitive individuals may be worsen by exposure to hot environment. Apparently, high environmental temperature raised the core temperatures of the heat exposed rats with consequent thermal dehydration. This possibly activated Renin angiotensin system (RAS) and sympathetic nervous system (SNS) to prevent against cardiovascular collapse (Gagnon et al., 2015).Indirect activation of the SNS could also result from angiotensin II, the effector of RAS (Tsuda, 2012) . The resultant activities of SNS increases cardiac contractility, cardiac output (Crandall and Wilson, 2015), vascular resistance (Kenney & Munce,2003) and renal Na+ retaining mechanism (Fujita, 2014) .These potential mechanisms possibly contributed to the observed elevated blood pressure , augmented heart rate and increased myocardial workload as reflected by RPP in this study.

In support of the elevated blood pressure and myocardial workload above, plasma sodium ion (Na+) increased significantly in the rats fed exclusively on high salt diet, in line with other previous investigations (Elias et al.,2011 & Oloyo et al., 2016) , and in the rodents exposed to hot environment alone, in support of studies by Allahverdi et al., 2013). The high plasma sodium in the rats fed a high salt diet is indicative of sodium retention, because under normal conditions 95% of ingested dietary sodium is expected to be excreted in urine (Law et al., 1991).

Similarly, this present study revealed that plasma sodium level was significantly much higher in the rats fed with high salt diet plus exposed to hot environment compared to rats fed a high salt diet alone. This indicates that high environmental temperature further raised plasma sodium level in the rats fed with high salt diet, in support of the exaggerated blood pressure and myocardial workload noticed in this group of rats. This

is evidently supported by the observation that plasma sodium clearance and urinary sodium excretion were significantly elevated in the salt loaded rats compared to the salt loaded rats concurrently exposed to hot environment. In other words, exposure to hot environment suppressed the hitherto elevated sodium excretion or natriuresis in salt loaded rats.

Evidently, in the face of exaggerated blood pressure, sodium excretion was suppressed in the salt loaded rats by exposure to hot environment, which is indicative of pressure –natriuresis resetting. Pressure-natriuresis is an important Physiological mechanism in which elevated arterial blood pressure results in increase renal sodium excretion or natriuresis (Roman, 1986). The mechanism works in a manner that accumulation of dietary sodium in body fluid, raises plasma osmotic pressure and promotes fluid retention, with resultant elevation of blood pressure towards the enhancement of renal sodium ion excretion. However, the resetting of this mechanism in most experimental and clinical hypertension maintains normal sodium excretion despite the elevation of arterial blood pressure. This is the picture seen in the salt-loaded animal models exposed to hot environment, as against the sustained higher sodium excretion observed in the exclusively salt-loaded rats in this study and in those of other studies (Oloyo et al., 2019).

The implication of this findings is that exposure to hot environment in salt-sensitive population may, promote remarkable rise in plasma sodium ion, via the resetting of pressure natriuresis. Consequently, this could translate into a severe form of salt sensitive hypertension, characteristic of the black population, living and working under high environmental temperature. In support of our result, high environmental temperature is known to cause thermal dehydration via sweating in human and excessive salivation in rodents, with initial loss of sodium and chloride ions. However, following acclimatization to chronic exposure to high environmental temperature, Renin Angiotensin System (RAS) is evidently activated to mitigate sodium and chloride ions loss (Wijkström et al., 2013).

Meanwhile, in this present study, plasma potassium ion (K+), chloride ion (Cl-) creatinine and urea as well as GFR were unaffected by exposure to hot environment in either the animals fed a high salt diet or in the animal fed on normal salt diet. This was similar for animals fed a high salt diet, although other investigators did suggest otherwise for urea and creatinine (Oloyo et al., 2016) . Nonetheless, our observation suggests that high salt diet or exposure to hot environment did not change K+, Cl- Creatinine and GFR either dependently or independently. This therefore clearly indicates that the

blood pressure and myocardial workload adjustment observed in this study is independent of GFR modulation (Hall et al.,1990) and unrelated to alteration in plasma potassium and chloride ions. In addition, the relatively normal plasma urea and plasma creatinine levels in this study suggest that renal function was relatively maintained, in the face of these two environmental stressors.

Next, our results show that elevated MAP, SBP, HR and RPP in the rats fed with high salt diet were not significantly reversed by treatment with telmisartan. This is in agreement with previous studies that demonstrated the limitation of telmisartan monotherapy to normalized MAP in spontaneous hypertensive rats(SHR) fed with high dietary salt (Susic et al., 2013). In a similar study, candesartan and losartan could not control hypertension in SHR fed with high salt diet, but attenuated the damaging organ effects of excess salt (Susic et al., 2010).

However, temisartan, effectively restored the severely elevated MAP, SBP, DBP and myocardial workload in the salt- loaded rats exposed to hot environment to a level comparable to control in this present study. Likewise, SBP and myocardial workload were significantly normalized by treatment with telmisartan in the rats exposed to hot environment alone. These findings indicate that salt-induced hypertension aggravated by hot environment may be mitigated by angiotensin II type I receptors blocker. This ultimately suggests a role for angiotensin II and its antagonist, in high environmental temperature influenced salt dependent and independent cardiovascular adjustment. In line with this reasoning, previous studies have suggested the need to downwardly review the doses of angiotensin receptor blockers in hot climate (Forsdyke, 2015) .

Furthermore, telmisartan did not alter plasma potassium, chloride ions, creatinine and GFR levels in all angiotensin II receptor blocker -treated experimental groups, but it attenuated plasma sodium level appreciably in rats fed with high salt diet under hot environment. However, this reduction did not reach a significant level. Similarly, sodium excretion and plasma sodium clearance were not significantly enhanced by temisartan in the rats exposed to hot environment plus fed a high salt diet, as well as in the rats fed a high salt diet alone or exposed to hot environment alone. This indicate that restoration of blood pressure, and myocardial workload by angiotensin II antagonism in the rodents chronically exposed to hot environment and high salt diet appeared to be independent of plasma potassium ions, chloride ions and GFR modulation. Nonetheless, it is not unlikely that telmisartan partly attenuated plasma sodium ion given the noticeable fall in plasma sodium ion with a corresponding slight increase in urinary sodium excretion in the telmisartan-treated animal models exposed to the combined environmental stressors.

Overall, our finding indicates that angiotensin type 1(AT1) receptor blocker possess antihypertensive potential that is likely influenced by environmental temperature. Ultimately, this potential could be beneficial in salt-induced hypertension under hot climate. The AT1 receptor mediated action was probably facilitated under these combined environmental factors. The possible actions of angiotensin II receptor inhibited by AT1 receptor blocker could include vasoconstriction, aldosterone release, catecholamine release, Arginine vasopressin peptide release, water intake and fluid retention (Barreras and Gurk-Turner, 2003).

In conclusion, blockade of AT1 receptor with telmisartan resulted in significant attenuation of the elevated SBP, DBP, MAP, and myocardial workload in salt loaded rats exposed to HET, with no reduction in rats fed with high salt diet alone. Our study indicates, for the first time that long term exposure to high environmental temperature significantly exaggerated cardiovascular response to high salt diet in salt loaded rats possibly via the angiotensin II type I receptor pathway. These findings provide a roadmap for future studies on the interaction between high environmental temperature and high salt diet in the pathophysiology and epidemiology of salt related hypertension and it potential cardiovascular outcome among the blacks. This study also provides a benchmark for the investigation of the therapeutic potentials or dilemma of angiotensin Receptor pathway under these dual environmental stressors.

ACKNOWLEDGMENTS

REFERENCES

Alam, M.M., Hashtimeem, M.A., Rahman, M.M., Hossain, M.M., Haque, M.R., Sobhan, Z., Islam, M.S., (2011). Effect of Heat Stress on Behavior, Physiological and Blood Parameters of Goat. Progressive Agriulture, 22(1 & 2): 37 – 45.

Allahverdi, A., Feizi, A., Takhtfooladi, M.A., Shabani, S., (2013). Effects of high environmental temperature on plasma sodium and potassium concentrations in commercial layers. European Journal of Experimental Biology. *3* (1): 592-595.

Amanda MacMillan, (2016). Global Warming 101 . https://www.nrdc.org/stories/global-warming-101 (accessed 8.21.19).

Barney, C.C., Kuhrt, D.M., (2016). Intermittent heat exposure and thirst in rats. Physiol Rep. 4(8): e12767.

Barreras, A., Gurk-Turner, C., (2003). Angiotensin II receptor blockers. Proc (Bayl Univ Med Cent). 16: 123–126.

Bernstein L, (2014). Salt intake is too high in 181 of 187 countries around the world - The Washington Post.htm, https://www.washingtonpost.com/news/to-your-health/wp/2014/08/14/salt-intake-is-too-high-in-181-of-187-countries-around-the-world.

Brook, R.D., Shin, H.H., Bard, R.L., Burnett, R.T., Vette, A., Croghan, C., Williams, R., (2011). Can Personal Exposures to Higher Nighttime and Early Morning Temperatures Increase Blood Pressure? J Clin Hypertens .13: 881–888.

Cappuccio, F.P., Kerry, S.M., Micah, F.B., Plange-Rhule, J., Eastwood, J.B., (2006). A community programme to reduce salt intake and blood pressure in Ghana [ISRCTN88789643]. BMC Public Health. 6, 13.

Cappuccio, F.P., Miller, M.A., (2016). Cardiovascular disease and hypertension in sub-Saharan Africa: burden, risk and interventions. Intern Emerg Med. 11: 299–305.

Crandall, C.G., Wilson, T.E., (2015). Human cardiovascular responses to passive heat stress. Compr Physiol. 5: 17–43.

Curtin, P.D., (1992). The slavery hypothesis for hypertension among African Americans: the historical evidence. Am J Public Health .82: 1681–1686.

Elias, S.O., Azinge, E.C., Umoren, G.A., Jaja, S.I., Sofola, O.A., (2011). Salt-sensitivity in normotensive and hypertensive Nigerians. Nig Q J Hosp Med. 21: 85–91.

Endo, S., Mori, T., Yoneki, Y., Nakamichi, T., Hosoya, Takuma, Ogawa, S., Tokudome, G., Hosoya, Tatsuo, Miyata, T., Ito, S., (2009). Blockade of angiotensin II type-1 receptor increases salt sensitivity in Sprague-Dawley rats. Hypertens. Res. 32: 513–519.

Forsdyke, D.R., (2015). Summertime dosage-dependent hypersensitivity to an angiotensin II receptor blocker. BMC Res Notes 8, 227. https://doi.org/10.1186/s13104-015-1215-8

Fujita, T., (2014). Mechanism of salt-sensitive hypertension: focus on adrenal and sympathetic nervous systems. J. Am. Soc. Nephrol. 25: 1148–1155.

Gagnon, D., Schlader, Z.J., Crandall, C.G., (2015). Sympathetic activity during passive heat stress in healthy aged humans. J Physiol. 593: 2225–2235. https://doi.org/10.1113/JP270162

Geng, Y., Zhu, L., Liu, F., Zhu, X., Niu, J., Li, G., (2016). Effect of dehydration heat exposure on thoracic aorta reactivity in rats. Biomed Rep. 5: 613–617.

Gobel, F.L., Norstrom, L.A., Nelson, R.R., Jorgensen, C.R., Wang, Y., (1978). The rate-pressure product as an index of myocardial oxygen consumption during exercise in patients with angina pectoris. Circulation. 57: 549–556.

Gohlke, P., Weiss, S., Jansen, A., Wienen, W., Stangier, J., Rascher, W., Culman, J., Unger, T., (2001). AT1 receptor antagonist telmisartan administered peripherally inhibits central responses to angiotensin II in conscious rats. J. Pharmacol. Exp. Ther. 298: 62–70.

Hajat, S., Vardoulakis, S., Heaviside, C., Eggen, B., (2014). Climate change effects on human health: projections of temperature-related mortality for the UK during the 2020s, 2050s and 2080s. J Epidemiol Community Health .68: 641–648.

Kenney W L and Munce TA .,(2003).Invited review: aging and human temperature regulation. J.Appl. Physiol. 95: 259-2603.

Lackland, D.T., (2014). Racial Differences in Hypertension: Implications for High Blood Pressure Management. Am J Med Sci. 348:135–138.

Law, M.R., Frost, C.D., Wald, N.J., (1991). By how much does dietary salt reduction lower blood pressure? I--Analysis of observational data among populations. BMJ .302: 811–815.

Lujan, H.L., DiCarlo, S.E., (2018). The "African gene" theory: it is time to stop teaching and promoting the slavery hypertension hypothesis. Advances in Physiology Education .42:412–416.

Malta, D., Petersen, K.S., Johnson, C., Trieu, K., Rae, S., Jefferson, K., Santos, J.A., Wong, M.M.Y., Raj, T.S., Webster, J., Campbell, N.R.C., Arcand, J., (2018). High sodium intake increases blood pressure and risk of kidney disease. From the Science of Salt: A regularly updated systematic review of salt and health outcomes (August 2016 to March 2017). J Clin Hypertens (Greenwich). 20: 1654–1665.

Mancia, G., De Backer, G., Dominiczak, A., Cifkova, R., Fagard, R., Germano, G., Grassi, G., Heagerty, A.M., Kjeldsen, S.E., Laurent, S., Narkiewicz, K., Ruilope, L., Rynkiewicz, A., Schmieder, R.E., Boudier, H.A.S., Zanchetti, A.,(2007). Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Journal of Hypertension 25, 1105–1187.

McLaren, C., Null, J., Quinn, J., (2005). Heat stress from enclosed vehicles: moderate ambient temperatures cause significant temperature rise in enclosed vehicles. Pediatrics .116: 109-112.

Meneton, P., Jeunemaitre, X., de Wardener, H.E., Macgregor, G.A., (2005). Links Between Dietary Salt Intake, Renal Salt Handling, Blood Pressure, and Cardiovascular Diseases. Physiological Reviews. 85: 679–715.

Mill, J.G., da Silva, A.B.T., Baldo, M.P., Molina, M.C.B., Rodrigues, S.L., (2012). Correlation between sodium and potassium excretion in 24- and 12-h urine samples. Braz J Med Biol Res .45: 799–805.

Moghadamnia, M.T., Ardalan, A., Mesdaghinia, A., Keshtkar, A., Naddafi, K., Yekaninejad, M.S., (2017). Ambient temperature and cardiovascular mortality: a systematic review and meta-analysis. PeerJ 5, e3574.

Mozaffarian D, Fahimi S, Singh GM, Micha R, Khatibz S, Engeli RE et al.,(2014).Global Sodium Consumption and Death from Cardiovascular Causes .
https://www.nejm.org/doi/full/10.1056/NEJMoa1304127 (accessed 8.20.19)

National Oceanic and Atmospheric Administration ,(2012). Excessive heat, a "silent killer" | National Oceanic and Atmospheric Administration https://www.noaa.gov/stories/excessive-heat-silent-killer (accessed 8.21.19).

National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, (2011). Guide for the Care and Use of Laboratory Animals, 8th ed, The National Academies Collection: Reports funded by National Institutes of Health. National Academies Press (US), Washington (DC).

Oloyo, A.K., Imaga, N.O.A., Fatope, Y., Sofola, O.A., (2019). Sex differences in cardiac and renal responses to a high salt diet in Sprague-Dawley rats. Heliyon 5. e01665.

Oloyo, A.K., Sofola, O.A., Anigbogu, C.N., (2011). Orchidectomy attenuates impaired endothelial effects of a high-salt diet in Sprague-Dawley rats. Can. J. Physiol. Pharmacol. 89, 295–304.

Oloyo, A.K., Sofola, O.A., Yakubu, M.A., (2016). Orchidectomy attenuates high-salt diet-induced increases in blood pressure, renovascular resistance, and hind limb vascular dysfunction: role of testosterone. Clin. Exp. Pharmacol. Physiol. 43: 825–833.

Roman, R.J., (1986). Pressure diuresis mechanism in the control of renal function and arterial pressure. Fed. Proc. 45, 2878–2884.

Sofola, O.A., Knill, A., Hainsworth, R., Drinkhill, M., (2002). Change in endothelial function in mesenteric arteries of Sprague-Dawley rats fed a high salt diet. J. Physiol. (Lond.). 543: 255–260.

Susic, D., Fares, H., Frohlich, E.D., (2013). Telmisartan prevents excess-salt-induced exacerbated (malignant) hypertension in spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. Ther. 18: 126–132.

Susic, D., Varagic, J., D Frohlich, E., (2010). Cardiovascular effects of inhibition of renin-angiotensin-aldosterone system components in hypertensive rats given salt excess. American journal of physiology. Heart and circulatory Physiology. 298: H1177-81.

Svetkey, L.P., Simons-Morton, D., Vollmer, W.M., Appel, L.J., Conlin, P.R., Ryan, D.H., Ard, J., Kennedy, B.M., (1999). Effects of Dietary Patterns on Blood Pressure: Subgroup Analysis of the Dietary Approaches to Stop Hypertension (DASH) Randomized Clinical Trial. Arch Intern Med. 159: 285–293.

Tsuda, K., (2012). Renin-Angiotensin System and Sympathetic Neurotransmitter Release in the Central Nervous System of Hypertension [WWW Document]. International Journal of Hypertension.

van de Vijver, S., Akinyi, H., Oti, S., Olajide, A., Agyemang, C., Aboderin, I., Kyobutungi, C., (2013). Status report on hypertension in Africa--consultative review for the 6th Session of the African Union Conference of Ministers of Health on NCD's. Pan Afr Med J. 16, 38.

Weinberger, M.H., Miller, J.Z., Luft, F.C., Grim, C.E., Fineberg, N.S., (1986). Definitions and characteristics of sodium sensitivity and blood pressure resistance. Hypertension 8, II127-134.

WHO, (2017). Cardiovascular diseases (CVDs). www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).

Wijkström, J., Leiva, R., Elinder, C.-G., Leiva, S., Trujillo, Z., Trujillo, L., Söderberg, M., Hultenby, K., Wernerson, A., (2013). Clinical and pathological characterization of Mesoamerican nephropathy: a new kidney disease in Central America. Am. J. Kidney Dis. 62:908–918.

Wilson, T.W., Grim, C.E., (1991). Biohistory of slavery and blood pressure differences in blacks today. A hypothesis. Hypertension. 17: I122-128.

Yamamoto, S., Iwamoto, M., Inoue, M., Harada, N., (2007). Evaluation of the effect of heat exposure on the autonomic nervous system by heart rate variability and urinary catecholamines. J Occup Health .49:199–204.