Niger. J. Physiol. Sci. 36 (December 2021): 149 –157
www.njps.physiologicalsociety.com
https://doi.org/10.54548/njps.v36i2.2

Niger. J. Physiol. Sci.

Full Length Research Article

Effect of Angiotensin Receptor Blockade on Plasma Osmolality and Neurohumoral Responses to High Environmental Temperature in Rats Fed a High Salt Diet

***Agbaraolorunpo F.M.[1], Oloyo A.K.[1], Ogunnowo S.A.[2], Anigbogu C.N.[1], Sofola O.A.[1]**

[1]*Department of Physiology, College of Medicine of the University of Lagos, Lagos, Nigeria;*
[3]*Department of Clinical Pathology of College of Medicine, University of Lagos, Nigeria*

Summary: Plasma osmolality (pOsmol) and neurohumoral signals play important roles in the pathophysiology of cardiovascular diseases. Our study investigated the effect of high environmental temperature (HET) on neurohumoral responses and pOsmol in rats fed a high salt diet (HSD), with and without angiotensin II receptor blockade (ARB), using telmisartan. Fifty-six male 8-week old Sprague-Dawley rats (95-110g) were randomly assigned into seven groups of 8 rats. These included control rats (I) fed with 0.3% NaCl diet (normal diet, ND); salt-loaded rats (II) fed with 8% NaCl (high salt) diet; ND rats (III) exposed to HET (38.5±0.5ºC) 4 hours daily per week; rats (IV) fed with 8% NaCl diet and exposed to HET daily. Others included rats (V) fed with 8% NaCl diet and treated with telmisartan (30mg/kg); ND rats (VI) exposed to HET and treated with telmisartan; rats (VI) fed with 8% NaCl diet, exposed to HET and treated with telmisartan. Plasma angiotensin II, aldosterone, vasopressin and norepinephrine (NE) concentrations were determined by ELISA technique; pOsmol from plasma K+, Na+ and Urea. HSD combined with HET in rats synergistically increased pOsmol ($P<0.001$) with an associated non-synergistic rise in fluid intake ($P<0.001$), fluid balance ($P<0.001$), plasma angiotensin II ($P<0.01$), aldosterone ($P<0.05$), NE ($P<0.001$) and vasopressin ($P<0.05$) concentrations compared to control. Telmisartan did not alter pOsmol in all the treated-rats, but normalized fluid intake levels and plasma vasopressin in the rats exposed to either HSD or HET *al*one. Prolonged exposure of rats to hot environment exacerbated the effect of excess dietary salt on pOsmol, with no effect on angiotensin II-mediated neurohumoral responses.

Keywords*: Plasma osmolality and neurohumoral responses to environmental heat and high dietary salt*

**Address for correspondence: fagbaraolorunpo@gmail.com +23408026441896*

Manuscript received- January 2021; Accepted- May, 2021

INTRODUCTION

Cardiovascular diseases are reported to be on the rise worldwide, accounting for over 30% of all global deaths (Benjamin *et al*., 2017), with the majority of the reported deaths in 2015 occurring in developing countries (WHO, 2017). High environmental temperature (Moghadamnia *et al*., 2017) and high dietary salt (Mozaffarian *et al*., 2015) are independent risk factors in the pathophysiology of cardiovascular diseases. Sadly, global warming is raising environmental temperature worldwide, consequently increasing the population of workers exposed to hot environment (Nerbass *et al*., 2017). High dietary salt consumption is also ascending in many nations of the world, including African countries (Cappuccio *et al*., 2006; Mozaffarian *et al*., 2015). Our previous work demonstrated that high environmental temperature increased the severity of hypertension in animal models fed a high salt diet for 8 week by exacerbating sodium retention in the animals (Agbaraolorunpo *et al*., 2019). This is important as blood sodium level is a principal determinant of blood osmolality critical to body fluid homeostasis and cardiovascular adaptation. Earlier studies showed that both high environmental temperature and high salt diet independently increased plasma osmolality (Suckling *et al*., 2012).This evidently drives the development, progression and outcome of cardiovascular and chronic kidney diseases (Kaya *et al*., 2017; Ozsari, 2017).

High environmental temperature causes sweating and dehydration that contract plasma volume and raises blood osmolality to stimulate thirst and antidiuresis responses (de Wardener *et al*., 2004). This feedback mechanism is critical for the restoration of body osmotic balance and fluid volume (Thornton, 2018). Precisely, plasma osmolality is controlled by homeostatic mechanisms located outside the blood brain barrier (Baylis & Thompson, 1988). These mechanisms involve central osmoreceptors located in the brain to monitor blood osmolality; thirst centre that mediate thirst response; and paraventricular nuclei (PVN) and supraoptic nuclei (SON) of hypothalamus which produce vasopressin. In support of this, it has been demonstrated that lesion of these areas of the brain abolishes both vasopressin secretion and thirst responses to hyperosmolality in both humans and experimental animals (Baylis & Thompson, 1988; Johnson & Thunhorst, 1997). In an attempt to restore plasma osmolality, vasopressin limits fluid loss from the sweat gland (Nadel, 1985) and kidney (Cuzzo *et al*., 2020). Again, it has been shown that thirst sensations occurs in response

to exaggerated plasma osmolality and angiotensin II, with conversely attenuated plasma volume (Hughes *et al*., 2018). The link between alterations in plasma osmolality and sympatHETic outflow has also been demonstrated in a study examining centrally located sensing mechanisms (Kinsman *et al*., 2017).

Meanwhile, angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor antagonists have both been shown to attenuate abnormal thirst drives (Sica, 2001). Likewise, blockade of angiotensin II type 1 receptors has been demonstrated to reduce water retention in dehydrated camels (Ali *et al*., 2012). But most worrisome, the efficacy of angiotensin II receptor blockade in the control of hypertension is marked with controversies with some study suggesting that administered alone, the drug is less effective among African population (Materson *et al*., 1993), ditto for ACE inhibitors (Brewster & Seedat, 2013).This dilemma may not be unconnected with the sensitivity of blood pressure to high salt diet among this race (Endo *et al*., 2009).This view is supported by the failure of angiotensin II blockers to control hypertension in salt-loaded animal models in our previous studies (Agbaraolorunpo *et al*., 2019) and other earlier studies (Susic *et al*., 2010). The physiological action of angiotensin II, such as sodium and water retention, aldosterone and vasopressin release is mediated by angiotensin type 1 receptor (AT1) receptor (Kaschina & Unger, 2003).Therefore, the inhibition of these receptor may modulate neurohumoral response to salt-induced and heat-induced alteration in plasma osmolality. This insight may help in further elucidating the plausible roles of plasma osmolality on blood pressure regulation by AT1 receptor blockade especially in individuals consuming high salt diets, with concomitant exposure to high environmental temperature.

MATERIALS AND METHODS

Animals: The study protocol was approved by the Ethics committee of College of Medicine of the University of Lagos (CMUL/HREC/11/18/471). Animal care and handling was done according to the National Research Council (US) Committee for the Care and Use of Laboratory (2011).

Fifty-six male Sprague-Dawley rats (8weeks, 95-110g) were supplied by Komad Farm, No 25 Old Abeokuta Lagos expressway. The rats were allowed to acclimatize for 2 weeks before the experimental procedures in standard cages at the animal house of Department of Physiology. The rats were maintained on a 12h dark/light cycle at ambient temperature of 25 ± 0.5 ^{o}C in the animal house. They were allowed access to standard rat chow and clean tap water ad libitum throughout the study.

Grouping of animals: The rats were randomly divided into 7 groups of 8 rats per group and subjected to different experimental conditions on the basis of dietary salt, environmental temperature and treatment with telmisartan (30mg/kg of animal's body weight).

Control (I): The rats were fed with normal salt diet (0.3% NaCl) and maintained at a room temperature of 25±0.5 ^{o}C for 8 weeks.

Salt (II): The rats were fed with high salt diet (8% NaCl) and maintained at a room temperature of 25 ± 0.5 ^{o}C for 8 weeks.

Heat (III): The rats were fed with normal diet (0.3%NaCl) and exposed to a high environmental temperature of 38.5 ± 0.5 ^{o}C 4 hours daily per week for 8 weeks.

Salt+Heat (IV): The rats were fed with high salt diet (8% NaCl) and exposed to a high environmental temperature of 38.5 ± 0.5 ^{o}C 4 hours daily per week for 8 weeks.

Salt+ARB (V): The rats were fed with high salt diet (8% NaCl), maintained at a room temperature of 25 ± 0.5 ^{o}C for 8 weeks and treated with telmisartan (30mg/kg per body weight) for 7 weeks.

Heat + ARB (VI): The rats were fed with normal diet (0.3%NaCl), exposed to a high environmental temperature of 38.5 ± 0.5 ^{o}C 4 hours for 8 weeks and treated with telmisartan (30mg/kg per body weight) for 7 weeks.

Salt+Heat+ARB (VII): The rats were fed with high salt diet (8% NaCl), exposed to a high environmental temperature of 38.5 ± 0.5 ^{o}C 4 hours daily per week for 8 weeks and treated with Telmisartan (30mg/kg per body weight) for 7 weeks.

Exposure of animals to high environmental temperature (HET): Heat-exposed rats were acclimatized to HET for one week starting from 30 ^{o}C to 35 ^{o}C with a daily temperature increase by 1^{o}C. Thereafter, the animals were exposed to HET at 38.5 ± 0.5^{o}C and relative humidity between 65 and 75 % using the method described by Barney & Kuhrt, (2016), with a slight modification of the temperature to a higher level of 38.5±0.5 ^{o}C. Exposure took place for 4 hours daily for 6 days/ week for 8 weeks in an environmental chamber from 9 a.m to 1p.m (Agbaraolorunpo *et al*., 2019). Environmental temperature was monitored with environmental thermometer. Rectal temperatures were determined, as index of core temperature, pre and post-exposure to HET with digital thermometer (Che Muhamed *et al*., 2016) .

Feeding of animals with high salt diet : The salt-loaded rats (Groups II, IV, V and VII) were fed with high salt diet as described by Sofola *et al*. (2002) for 8 weeks.

Treatment of animals with Angiotensin II receptor blocker: ARB-treated groups (V, VI and VII) were administered with 30 mg/kg/day telmisartan daily via oral gavage (Gohlke *et al*., 2001) for 7 weeks, starting from the 2nd week of the experiment. The volume dose was calculated as Volume = $\frac{dose(mg/kg) * weight\ of\ rat(kg)}{conc\ (mg/ml}$

The telmisartan (MSN Laboratory, India) was procured from Phillips Pharmaceuticals Ltd, Nigeria.

Blood collection: Retro-orbital puncture was performed on the experimental rats for the collection of blood sample. The samples were collected in heparin bottles. The blood samples were centrifuged at 3000 rpm for 15 minutes to separate plasma from whole blood. Plasma samples were kept in eppendorf tubes and stored at -25 ^{o}C in refrigerator until the assays of the respective peptides.

Urine collection: Twelve-hour urine output (V) in mls was determined at the end of 8th week of the experiment, from 7 p.m to 7 a.m. in a metabolic cage. Plasma Na^{+} in mmol/L was determined with ion selective electrode method (ISE 6000 analyzer, France), while plasma urea (mg/dl) was determined colorimetrically. Plasma osmolality was

determined by a method validated by (Martín-Calderón *et al.*, 2015). Net fluid gain was calculated by subtracting total urine loss from water intake, assuming that respiratory water loss and sweat loss at rest were negligible (Hiroshi *et al*., 1994).

Measurements of plasma Angiotensin II, Aldosterone, Norepinephrine, Arginine Vasopressin

Plasma Angiotensin II: Plasma angiotensin II concentrations were determined using Rat angiotensin II ELISA kit (MyBiosource.com, USA) according to the manufacturer's instruction. Briefly, 50µl standard was added to standard micro elisa plates, while 10 µl of plasma was added to testing well which was followed by the addition of 40 µl of sample diluent into plasma. 100µl of HRP-conjugate was added to each well (standard well and testing) and incubated at 37 ºC for 60 minutes. Thereafter, the plates were washed 5 times, followed by the addition of 50 µl of chromogen solution A and B respective at 37 ºC within 15 minutes in the dark for colour development (blue).50µL stop solution was then added into each of well to stop the reaction, resulting in colour change from blue to yellow. The absorbance was read at 450 nm in microplate within 15 minutes of adding the stop solution which was used to determine the concentration of angiotensin II.

Plasma Aldosterone: Plasma aldosterone concentrations were determined using Rat Aldosterone ELISA kit (MyBiosource.com, USA) according to the manufacturer's instruction. Briefly, 50 µl standard was added to standard microelisa plates, while 10µl of plasma was added to testing well which was followed by the addition of 40 µl of sample diluent into plasma. 100 µl of HRP-conjugate was added to each well (standard well and testing) and incubated at 37 ºC for 60 minutes. Thereafter, the plates were washed 5 times, followed by the addition of 50 µl of chromogen solution A and B respective at 37 ºC within 15 minutes in the dark for colour development (blue). A stop solution (50 µL) was then added into each of well to stop the reaction, which changed the colour from blue to yellow. The absorbance was read at 450nm in microplate within 15 minutes of adding the stop solution which was used to determine the concentration of aldosterone.

Plasma Norepinephrine: Plasma Norepinephrine concentrations were determined using Rat Norepinephrine ELISA kit (MyBiosource.com, USA) according to the manufacturer's instruction. Briefly, 50µl standard was added to standard micro elisa plates, while 10 µl of plasma was added to testing well which was followed by the addition of 40 µl of sample diluent into plasma. 100 µl of HRP-conjugate was added to each well (standard well and testing) and incubated at 37ºC for 60 minutes. Thereafter, the plates were washed 5 times, followed by the addition of 50 µl of chromogen solution A and B respective at 37 ºC within 15 minutes in the dark for colour development (blue). A stop solution (50 µL) was then added into each of well to stop the reaction, which changed the colour from blue to yellow. The absorbance was read at 450 nm in microplate within 15 minutes of adding the stop solution which was used to determine the plasma concentration of Norepinephrine.

Plasma Arginine vasopressin: Plasma vasopressin concentrations were determined using Rat Vasopressin ELISA kit (BioAim Scientific Inc, Canada) according to the manufacturer's instruction. 25 µl of ant-vasopressin antibody was added into all the wells.75 µl of assay diluent were pipetted into blank wells.50µl of assay diluent into 0 ng/ml standard.50µl and other graded preparation of standard solution.50 µl each of plasma samples were added to appropriate wells. 25 µl of biotinylated peptides was added into each well (except the blank well), sealed and incubated for 1 hour at room temperature The well was emptied and the content washed with buffer of 300µl four times.100µl of diluted streptavidin-HRP solution was added into each well, followed by the sealing of the well and incubation of the wells at room temperature for 45minutes, while gently shaking the wells. The washing of the wells with buffer solution was repeated as previously done.100 µl of TMB substrate solution was added into each well and incubated for 15 minutes at room temperature with gentle agitation in the dark to allow for colour development.50 µl of stop solution was added to the well to terminate the reaction. The absorbance in each well was read immediately within 30 minutes of stopping reaction and this was used to determine the concentration of the samples.

Statistical analysis: Data were presented as Mean ± SEM. Differences in experimental rat groups were compared with one-way ANOVA followed by Tukey post-hoc test. P<*0.05* was regarded as statistically significant. GraphPad 5 software package (GraphPad Software, California ,USA) was used for the analysis

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RESULTS

Influence of High Environmental Temperature (HET) on plasma osmolality (pOsmol) in salt-loaded rats (HSD) treated with ARB: In comparison with control rat group, pOsmol was significantly higher in the rat group fed a HSD (P<0.01), the group exposed to HET *al*one (P<0.05), as well as the group exposed to the combined factors (P< 0.001). Meanwhile, pOsmol was significantly higher in the rat group exposed to the combined environmental factors compared with the rats fed with HSD alone (P<0.05) and in the group exposed to HET *al*one (P<0.05). Meanwhile, ARB did not significantly moderate pOsmol in all the experimental rat groups (P>0.05) (Figure 1).

Influence of High Environmental Temperature on fluid intake, fluid balance and urine output in salt-loaded rats treated with ARB: In comparison with control rat group, fluid intake (Table 1) and fluid balance respectively (Figure 2) were significantly increased in rat group fed with high salt di*et al*one (P<0.01 and P<0.01) and in the group exclusively exposed to high environmental temperature (P< 0.001 and P<0.001) as well as in the group exposed to the two environmental factors (P< 0.001 and P< 0.001). Meanwhile, angiotensin II receptor blockade in the salt-loaded rat group, and in the group exposed to high environmental temperature, significantly reduced the salt-induced and the heat-induced rise in fluid intake (P<0.05 and P<0.001 respectively), ditto for the attenuation of the rise in fluid balance (P<0.05 and P<0.001 respectively).

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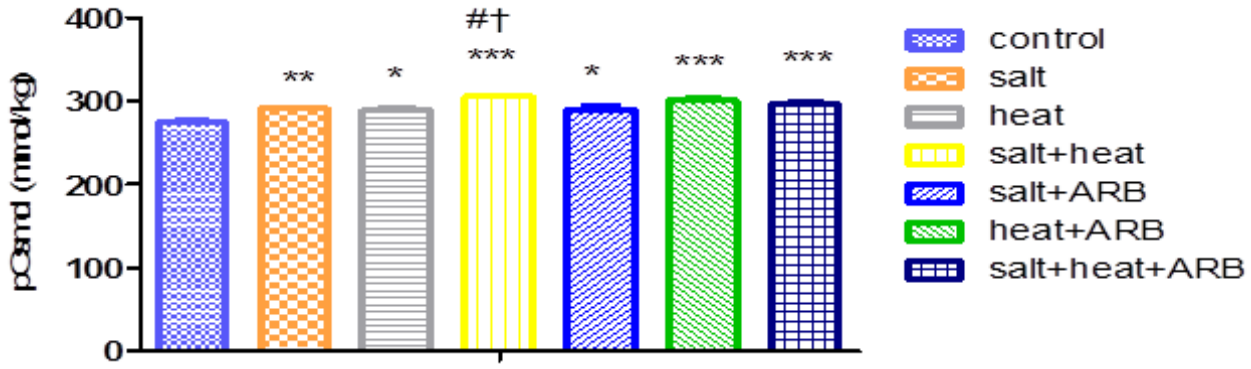

Figure1:
Plasma Osmolality in salt-loaded rats exposed to HET with and without ARB treatment.
*P<0.05, **P<0.01, ***P<0.001 vs control; #P<0.05 vs salt; †P<0.05 vs heat (n≥6).

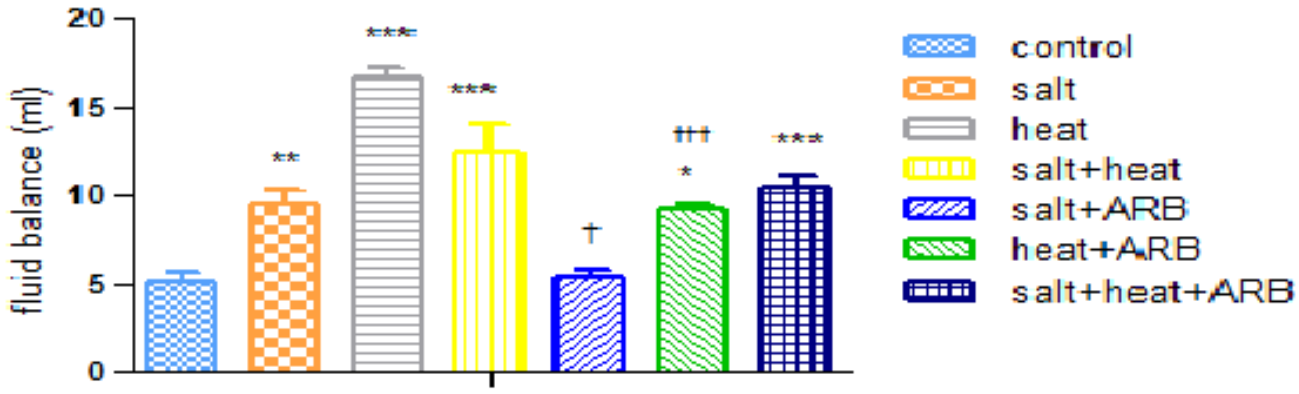

Figure 2:
Fluid balance in salt-loaded rats exposed to HET with and without ARB treatment:
**P<0.01, ***P<0.001 vs control; †††P<0.001 vs heat; † P<0.01 vs salt. Data presented as Mean ± SEM; One-way ANOVA followed by Tukey multiple comparison test (n≥7).

Table 1:
Effect of HET on Fluid intake and urine output in salt-loaded rats treated with and without ARB

Group	Fluid Intake (ml)	12-Urine output (ml)
control	9.6 ± 0.9	3.9 ± 0.7
Salt	18.04 ±1.1**	7.2± 0.8**
Heat	20.3 ±1.1***	2.2 ± 0.4##
Salt+Heat	20.0 ±1.3***	5.1 ± 0.8#
Salt+ARB	13.5± 0.3†	4.8 ± 0.3†
Heat+ARB	9.6 ±0.3†††	0.5 ± 0.2
Salt+Heat+ARB	15.5 ±1.3**†	3.4 ± 0.7

Fluid Intake: ***P<0.01,***P<0.001 vs control, † P<0.05 vs Salt , †† P<0.01 vs Heat;* ***Urine output:*****P<0.01 vs control, ##P<0.01 vs salt; Data presented as Mean ± SEM; One-way ANOVA followed by Tukey multiple comparison test (n≥7). Keys: HET, high environmental temperature; ARB, angiotensin receptor blocker.*

In contrast, ARB did not significantly attenuate fluid balance in the salt-loaded rats that were exposed to high environmental temperature, although it reduced their fluid intake significantly.
Furthermore, urine volume was significantly higher in rats fed a HSD compared to control rats (P<0.01) but this was attenuated in the ARB-treated rat fed a HSD (P<0.05). However, rats exposed to HET *al*one and combined with HSD had no change in their urine volume compared to control rats, ARB showed no modulatory effect under these conditions (Table 1).

Furthermore, urine volume was significantly higher in rats fed a HSD compared to control rats (P<0.01) but this was attenuated in the ARB-treated rat fed a HSD (P<0.05). However, rats exposed to HET *al*one and combined with HSD had no change in their urine volume compared to control rats, ARB showed no modulatory effect under these conditions (Table 1).

Influence of High Environmental Temperature on Neurohumoral responses in salt-loaded treated with rats with ARB: Rats exposed to HET *al*one had significant increase in plasma angiotensin II (P<0.05), aldosterone (P<0.001), arginine vasopressin (AVP) (P<0.001) and norepinephrine (P<0.001) compared with control rats. Similar increases were recorded in plasma angiotensin II (P<0.05), aldosterone (P<0.05), AVP (P<0.05) and norepinephrine (P<0.001) concentrations in the rats exposed to HET combined with high salt diet. However, only angiotensin II (p<0.05) and aldosterone (p<0.01) were

significantly elevated in the rats fed with high salt di*et al*one. Meanwhile, plasma angiotensin II, aldosterone, and AVP concentrations in the three groups of ARB-treated rats were comparable to the respective plasma levels in the control rats. Paradoxically, the increase in plasma norepinephrine was maintained in the ARB-treated rats fed with high salt diet ($P<0.05$) and in the rats exposed to HET ($P<0.05$) respectively compared with the control rat group. However, ARB normalised plasma aldosterone ($P<0.05$) and AVP ($P<0.01$) in the rats exposed to HET *al*one compared with the untreated rats in similar condition. AVP was similarly maintained at normal level ($P<0.01$) in the rats exposed to the combined factors compared with the untreated rats (Table 2).

Table 2:
Neurohumoral responses to dietary salt and environmental heat with and without ARB

Groups	Angiotensin II(pg/ml)	Aldosterone (pg/ml)	AVP(pg/ml)	NE(ng/ml)
control	619.3 ± 8.0	253.8 ± 15.9	1.80 ± 0.3	4.5 ± 0.4
salt	692.6 ± 12.7*	334.5 ± 13.3**	2.76 ± 0.6	5.6 ± 0.3
heat	675.6 ± 9.0*	353. ±18.5***#	5.4 ± 0.8***	9.2 ± 0.6***##
Salt+heat	692.9 ± 19.0**	320.1 ± 14.6*	4.16 ± 0.3*	9.9 ± 0.3***##
Salt+ARB	630.7±5.6	304.6 ± 7.9	2.33 ± 0.3	7.5 ± 1.2*
heat+ARB	625.0 ± 9.4	291.1 ± 13.7†	2.53 ± 0.3††	7.4 ± 0.6*
Salt+heat+ARB	673.6 ± 19.4	288.8 ± 4.8	2.06 ± 0.2†	6.2 ± 0.8††

Aldosterone**: *P<0.05,**P<0.01,P<0.001 vs control; #P<0.05 vs salt ;†P<0.05 vs heat ;*
AVP: ***P<0.05,P<0.001 vs control, †P<0.05 vs salt + heat, ††P<0.01 vs heat; **Norepinephrine**:*P<0.05,***P<0.001 vs control,##P<0.01 vs salt ,††P<0.001 vs salt + heat, ††. One-way ANOVA, followed by Tukey multiple comparison test (n ≥6)*

DISCUSSION

This present study investigated the effect of chronic exposure to high environmental temperature (HET) on plasma osmolality (pOsmol) and neurohumoral responses in rat models fed with high salt diet in the presence and absence of angiotensin receptor blocker (ARB), telmisartan. Our results revealed that chronic consumption of high salt diet (HSD) and prolonged exposure to high environmental temperature (HET) respectively and together increased pOsmol in our animal models. But most importantly, HET and HSD combined together in our experimental rats resulted in pOsmol level higher than that in either of the rats fed a HSD alone or exposed to HET *al*one. Surprisingly, angiotensin II receptor blockade, using telmisartan did not reverse the rise in pOsmol under the individual environmental condition as well as under the combined environmental conditions.

Our result is in line with previous study which showed that high salt diet increased pOsmol (Suckling *et al*., 2012), ditto for high environmental temperature (Gagnon *et al*., 2017). Most importantly, our study for the first time demonstrated the possible synergistic effect of these dual environmental factors on plasma osmolality when combined. This synergy possibly explains in part a probable mechanism that contributed to the exaggerated blood pressure and myocardial workload observed in our experimental animals fed a high salt diet in combination with exposure to high environmental temperature as reported in our previous work (Agbaraolorunpo *et al*., 2019).This view is supported by earlier studies which showed that increased blood osmolality resulted in elevated mean arterial pressure and heart rate (Gagnon *et al*., 2017; Kanbay *et al*., 2018). Furthermore, plasma sodium ion which has a direct relationship with high salt diet (Oloyo *et al*., 2016) and high environmental temperature (Allahverdi *et al*., 2013), possibly contributed to the increased plasma osmolality in this study. The increased plasma osmolality, otherwise referred to as hyperosmolality, when detected by SFO (subfornical organ) and OVLT (organum vasculosum) in the brain, could trigger series of homeostatic responses, including thirst (Hughes *et al*., 2018), sympatHETic nervous system activation and arginine vasopressin release (Leib *et al*., 2016), critical to body fluid volume expansion and blood pressure maintenance.

Therefore, the combined action of HSD and HET on pOsmol was expected to produce a corresponding synergistic effect on body fluid balance by increasing fluid consumption and reducing urine output. But our results revealed otherwise, showing that HSD and HET interactively potentiated fluid consumption and fluid balance, similar to the increases caused by either HSD (Bankir *et al*., 2017) or HET (Cuzzo *et al*., 2020), without any synergistic association. In agreement with earlier studies (Eriksson *et al*., 1984, Denton *et al*., 1985), urine output was also increased by HSD alone, but combined with HET, the increase was cancelled out in our animal models. These observations suggest that high environmental temperature possibly possess the potential to blunt salt-induced renal fluid loss, and this may play an important role in fluid retention. Interestingly, telmisartan normalized the increased fluid intakes in rat group exposed to the individual environmental factors and in the group exposed to the combined factors. Similarly, telmisartan significantly normalized the rise in fluid balance in the rats fed with excess salt diet and in the rats exposed to high environmental temperature respectively, with slight effect in the rats exposed to the combined environmental factors. This finding suggests a possible role for AT1receptor on thirst response and fluid balance adjustment (Sica, 2001) to the observed hyperosmolality caused by high salt diet and high environmental temperature respectively and together.

This plausible action of AT1 receptor blocker on body fluid homeostasis may in turn be modulated cardiovascular responses under these environmental conditions.

Evidently, the compensatory fluid volume adjustment to high salt diet and high environmental temperature was mediated by neurohumoral factors. These factors include angiotensin II, involves with thirst (Fitzsimons, 1998) and AVP release (Szczepanska-Sadowska *et al*., 2018); aldosterone, involves with renal sodium retention (Mulrow, 1999) and AVP, involves with fluid retention in the kidneys (Cuzzo *et al*., 2020) and sweat glands (Nadel, 1985). Hyperosmolality is also reported to increase sympatHETic nerves activity through osmosensitive neurons located in the forebrain circumventricular organs (Toney *et al*., 2003). Meanwhile, our current result showed that plasma angiotensin II and aldosterone levels were raised in the rats fed with high salt diet, similar to the inappropriate activation of these hormones reported by Gonsalez *et al*. (2018). But this was in disagreement with the suppressed levels of these hormones reported by Stocker *et al*., (2003) and Ramachandran *et al*. (2019).

Increases in circulatory angiotensin II and aldosterone were also noticed in the rat group exposed to HET in line with previous studies (Kosunen *et al*., 1976; Ma *et al*., 2001). Likewise, rats exposed to the combine environmental factors (HSD and HET) showed similar increases in angiotensin II and aldosterone, devoid of synergistic effect. These increases in circulatory angiotensin II and aldosterone were however less pronounced in the telmisartan-treated rats exposed to either of the individual factors or the combined factors. This finding indicates that ARB therapy moderately blunted angiotensin II and aldosterone responses in the rat groups fed with either HSD alone or combined with HET. This present finding contradicts previous results from earlier studies that reported increases in circulatory angiotensin II, following a prolonged therapy with angiotensin II receptor blockade (Nussberger *et al*., 1986; van den Meiracker *et al*., 1995). However, our present result aligned with findings from other works which demonstrated that long term AT1 receptor blocker therapy in hypertensive patients decreased plasma angiotensin II and aldosterone (Ichihara *et al*., 2001; Agata *et al*., 2006).

Furthermore, circulatory AVP and norepinephrine were significantly increased in the rats exposed to HET *al*one and combined with high salt diet, with no synergistic effect observed. Noticeably, high salt di*et al*one did not significantly increase AVP and norepinephrine in agreement with a study by Block *et al*., (1984), but contrary to studies by Kjeldsen *et al*., (1985) and Campese *et al*. (1982). Telmisartan did not attenuate the elevated norepinephrine level in the rats exposed to HET *al*one, but normalized AVP level. In contrast, telmisartan attenuated the increased plasma norepinephrine and AVP level in the rat group exposed to combined environmental factors, but paradoxically exaggerated the hitherto unchanged norepinephrine level in the group of rats fed a high salt di*et al*one. This finding suggests that interaction between high salt diet and high environmental temperature possibly improves AT1 receptor response to ARB therapy under the aforementioned condition. Given the roles play by norepinephrine (Kasparov & Teschemacher, 2008) and AVP (Matsuhisa *et al*.,2000) in the development of essential hypertension, it is not impossible that the effective blockade of the release of these two important peptides contributed to the effective blood pressure control in the rat group fed with high salt diet in combination with exposure to high environmental temperature as reported in our earlier work (Agbaraolorunpo *et al*., 2019).

Generally, blockers of AT1 receptor possess antiadrenergic potential to attenuate catecholamine level (Diz *et al*., 201; Balt *et al*., 2002). In support of this, earlier investigation revealed that AT1 receptor antagonist blunted Central Nervous SympatHETic activity in experimental animals with heart disease (Ramachandran *et al*., 2019). Averill *et al*. (1994) also demonstrated that losartan, an AT1 receptor antagonist, attenuated pressor and sympatHETic overactivity induced by angiontensin II in spontaneous hypertensive rats. Perhaps, the exaggerated plasma level of norepinephrine in the ARB-treated rats, fed a high salt di*et al*one, partly explains the unresponsiveness of salt-induced hypertension to ARB therapy in our previous study (Agbaraolorunpo *et al*., 2019) and in a study by Endo *et al*. (2009).

Furthermore, the increased plasma angiotensin II observed in this study likely promoted fluid intake and fluid gain in the hyperosmotic state of the rats fed a high salt diet and exposed to high environmental temperature respectively and together. This is supported by the attenuation of fluid intake by AT1 receptor blockade with telmisartan under this hyperosmotic condition. Similarly, Angiotensin-converting enzyme (ACE) inhibitors and Angiotensin II type 1 receptors antagonists have also been shown to attenuate abnormal thirst drives (Sica .,2001). Likewise, blockade of angiotensin II type 1 receptors was suggested to compromise water retention in a dehydrated camel (Ali *et al*., 2012). Therefore, it is not impossible that central inhibition of thirst and vasopressin by AT1 receptor inhibitor plays an active role in this process as suggested by our results. This is in conformity with earlier studies by Gohlke *et al*. (2002) and Nishimura *et al*., (2000) which demonstrated that peripherally administered ARB therapy sufficiently blocks centrally mediated action of angiotensin II.

Meanwhile, the rise in circulatory aldosterone associated with increased circulatory angiotensin II in this current study apparently promoted sodium ion retention. This ultimately contributed to the hyperosmolality caused by high salt diet and high environmental temperature. Although, the response of aldosterone was blunted by angiotensin receptor inhibition in line with a study by Nakamura *et al*.(2014), this action did not alter hyperosmolality in all the experimental rats treated with telmisarn as anticipated. This could be due to the attenuation of circulatory AVP level by ARB therapy, with the consequent suppression of AVP's antidiuretic action. This possibly compromised body fluid gain needed to normalize the observed hyperosmolality. The decline in circulatory AVP in this current study, was apparently counteracted by enhanced renal responsiveness to AVP as suggested by the decline in urine output in the telmisartan-treated rats. This could be a possible novel renal-defence mechanism geared towards ensuring fluid balance in the face of diminished thirst and plasma AVP possibly engendered by ARB therapy in a dehydrated state. This is supported by a study which proposed that renal action of circulatory AVP varies,

depending on the presence or absence of endogenous prostaglandin (Usberti *et al*., 1985).

Overall, chronic exposure to high environmental temperature synergized with high salt diet in our experimental rats to raise plasma osmolality, with the corresponding activation of fluid-regulating neurohumoral factors. Although, the combined effect of high salt diet and high environmental temperature on neurohumoral responses and body fluid balance was not additive, angiotensin II receptor blockade blunted these responses without correcting the exaggerated hyperosmolality. Therefore, patients on AT1 receptor blockers must be encouraged to take adequate water, given the tendency of this class of drug to blunt thirst and compromise body fluid balance with consequent hyperosmolality.

Acknowledgement

The Authors wish to acknowledge the contribution of Mr Afolabi O.O of the Animal House of College of Medicine of the University of Lagos Physiology and Mr Otta Duncan Azubuike of the Department of Physiology of the College of Medicine for the animal care and technical support

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