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Research article

Synergistic Vascular Response to Combined Effect of High Salt Diet and High Environmental Temperature Mitigated by Angiotensin II Receptor Blockade

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ABSTRACT

This study investigated the plausible role of angiotensin II receptor on vascular mechanism underlying the pathophysiology of salt-induced hypertension in rats exposed to high environmental temperature chronically. Aortic rings were obtained from seven groups of male Sprague Dawley rats (n=6) including control rats (I) fed with 0.3% NaCl diet (normal diet, ND); salt-loaded rats (II) fed with 8% NaCl high salt diet (HSD); ND rats (III) exposed to HET (38.5±0.5 ºC) 4 hours daily per week; rats (IV) fed with 8% NaCl diet and exposed to HET daily; rats (V) fed with 8% NaCl diet and treated with telmisartan (30mg/kg); ND rats (VI) exposed to HET and treated with telmisartan; rats (VII) fed with 8% NaCl diet, exposed to HET and treated with telmisartan. Photomicrography study was conducted on the first set of rings and the second set of rings were pre-contracted with Norepinephrine (NE) at 10-4 or 10-5, M to obtain a maximum peak contractile response, followed by the assessment of vascular relaxation response to graded doses of acetylcholine (Ach) and sodium nitroprusside (SNP) respectively from10 -9 to 10-4 M in endothelial intact aortic rings. Vascular relaxation to Ach and SNP were impaired in rings of rats fed a HSD and exposed to HET respectively and combined, with non-synergistic response. But in contrast, contractile response to NE in vessels with combined exposure was synergistic, but mitigated by telmisartan, an angiotensin II receptor blocker, ditto for the photomicrograph of the vessels. Angiotensin II blockade with telmisartan partly mitigated the deleterious vascular impact of combined exposure to high salt diet and environmental heat.

Keywords: *Telmisartan, vascular response, angiotensin receptor blocker, high salt, chronic heat exposure*

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INTRODUCTION

Hypertension has been identified as a leading cause of cardiovascular diseases globally (Lozano *et al*., 2012). It is also a major cause of morbidity and mortality in most nations of the world (Mozaffarian *et al*., 2015). In particular, the burden of hypertension is high and progressing among, African population (Okello *et al*., 2020). Vascular mechanisms play pivotal roles in blood pressure regulation. These mechanisms maintain a balance between vasoconstriction and vasodilation. While vasoconstriction is mediated by intrinsic myogenic factors and vasoconstrictors such as norepinephrine, epinephrine and angiotensin II, vasodilatation is mediated by endothelial dependent and independent mechanisms. The endothelial dependent mechanism is driven by endothelial dependent relaxation factor (EDRF), which has the same physiochemical properties as nitric oxide (NO), synthesized by Nitric oxide synthase (NOS). The main target of NO in smooth muscle cells is soluble guanylyl cyclase, which increases cGMP (Hofmann *et al*., 2006). In turn, the cGMP mediates endothelial independent vascular relaxation.

Endogenously, acetylcholine act via NOS in intact endothelium to promote the release of NO. But pharmacological agents such as glyceryl trinitrate (GTN) and sodium nitroprusside (SNP) exert their effects via cGMP-dependent mechanisms (Torfgård & Ahlner, 1994). The disruptions of these mechanisms, characterized by exaggerated vascular response to endogenous vasoconstrictors and reduced response to vasodilators, play major roles in the pathogenesis of hypertension (Sofola *et al*., 2002). Most importantly, these changes may be associated with hypertrophy of vascular smooth muscles (Oloyo *et al*., 2011). The work of dos Santos *et al*., (2006) suggests that salt-induced increment in vascular reactivity is endothelium-dependent mediated by the activation of local Renin Angiotensin System, with Angiotensin II receptor blocker reported to improve endothelial function by reducing ROS production (Nurkiewicz *et al*., 2010).

High salt diet plays a critical role in the development of hypertension, especially among people of colour of African extraction. Disruption of vascular mechanism is one of the pathophysiology through which high salt diet engenders blood pressure rise. For example, Bragulat *et al*. (2001) demonstrated the impairment of ACh-induced vasodilation in the pathogenesis of salt sensitive hypertension. Our authors have also previously demonstrated that prolong exposure to hot environment worsened the severity of salt-induced hypertension, using animal model (Agbaraolorunpo *et al*., 2019). We therefore hypothesized that vascular mechanism plays a role in this synergistic effect of high salt diet and high environmental temperature on the severity of the salt-induced hypertension in our previous work, with angiotensin II type receptor pathway occupying a central position.

MATERIALS AND METHODS

Experimental animals: Animal care and handlings were done according to the National Research Council (US) Committee for the Care and Use of Laboratory Animals (National Research Council (US) Committee for the Update of the Guide 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). Forty-two male Sprague-Dawley rats weighing between 95 and 110 g were used at each phase of the study. The rats were maintained on a 12h dark/light cycle at 25 ± 0.5 oC room temperature in the animal house and were allowed access to standard rat chow and clean tap water ad libitum throughout the study. The rats were randomly divided into experimental groups with 6 rats per group. The study was performed at the Department of Physiology Research Lab of the College of Medicine, University of Lagos.

Experimental Animal grouping

Allocation of animals into experimental groups: The rats were randomly assigned into following experimental group for 8 weeks (n=6)

Group I: Control rats, fed with normal rat chow (0.3 % NaCl) and kept at room temperature.

Group II: Salt-loaded, fed with High salt diet (8% NaCl) and kept at room temperature.

Group III: Heat-exposed, fed with normal rat chow (0.3 % NaCl) and exposed to HET (38.5 ± 0.5 oC)

Group IV: Salt + Heat, fed with High salt diet (8% NaCl) and exposed to HET (38.5 ± 0.5 oC)

Group V: Salt + ARB, Salt-loaded, fed with High salt diet (8% NaCl) and kept at room temperature, with daily oral telmisartan (30mg/kg) treatment.

Group VI: Heat + ARB, Heat-exposed, fed with normal rat chow (0.3 % NaCl) and exposed to HET (38.5 ± 0.5 oC), with daily oral telmisartan (30mg/kg) treatment.

Group VII: Salt + Heat + ARB, fed with High salt diet (8% NaCl) and exposed to HET (38.5 ± 0.5 oC), with daily oral telmisartan (30mg/kg) treatment.

Telmisartan was administered from 2nd -8th experimental week.

Vascular Reactivity to Norepinephrine, Acetylcholine and Sodium Nitroprusside: The experimental rats were sacrificed via cervical dislocation which is fast, painless and preferred for terminal vascular study. The abdomen was opened up and the abdominal aorta isolated and cut into rings of 2-3mm in cold Physiological Salt Solution (PSS) maintained at 4 oC. are was ensured to avoid contact with the endothelial surface during the removal and the mounting of the vascular rings. The PSS consisted of 119.0 mol/L of NaCl, 4.7 mol/L of KCL, 1.2 mol/L of KH2PO4, 1-2 mol/L of MgSO4, 24.9 mol/L NaHCO3, 1.6 mol/L CaCl2 and 11.5 mol/L glucose (Oloyo *et al*., 2011). The rings were subsequently mounted on pressure transducer suspended in organ bath, containing PSS, maintained at PH of 7.35-7.45, temperature of 37 o.C and perfused with 95% O2: 5% CO2 mixture. After mounting the rings, a passive tension of 1.5 g was applied to each ring, and the rings were then allowed to equilibrate in the PSS for 90 minutes. Then the suspended aorta rings in the organ bathe were charged with norepinephrine at 30 minutes interval for 90 minutes. This was followed by the evaluation of vascular responses to graded concentration of NE fom10 -9 to 10-4 M.

In separate experiment, the rings were washed and pre-contracted with NE at 10-4 or 10-5 , M to obtain a maximum peak contractile response, followed by the assessment of vascular relaxation response to graded doses of ACh and SNP receptively from10 -9 to 10-4 M in endothelial intact aortic rings. The vascular tension responses to the graded concentration of agonist from 10 -9 to 10-4 M were represented as percentage responses. The log dose-response curve was plotted for the contractile and relaxation responses. LogE50 and % maximum concentration among the groups were presented as Mean ± SEM and compared with One-way ANOVA.

Plasma nitric oxide measurement: Blood was collected via cardiac puncture into heparinized bottles and centrifuged at 3000 rpm for 15 minutes to extract plasma samples for nitric oxide (NO) concentrations determination. Total plasma concentration of Nitrites (NO2-) and Nitrate (NO3-) were used as indicators of plasma Nitric oxide (Moshage *et al*., 1995). The Griess reaction (Sun *et al*., 2003) was used for the assay. The Griess reaction, also called diazotization assay, is based on the conversion of nitrite to a purple-coloured azo-dye that can be spectrophotometrically assayed at a wavelength of ~540 nm (Csonka *et al*., 2015).

Photomicrography of aortic vessel rings: Abdominal aorta tissues were harvested from the experimental rats after the animals were sacrificed. The tissues were fixed in 10 % of formaldehyde, and this was followed by serial dehydration of tissues in 60 - 70 % ethanol, progressing through 90-95 % ethanol and a later clearance in xylene solution. The tissues were then embedded in paraffin wax. This was followed by tissues sectioning with microtomes and placement of sections on slides for staining with masson's trichrome and counter staining with light green stain for the enhancement of vascular collagen. The sections were viewed with microscope (CELTECH BL-1000, China), while the vessel's wall

thickness was measured from it calibrated callipers in the microscope.

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. Graph pad 5 software package (GraphPad Software, California, USA) was used for the analysis. Statistical significance set at P<0.05.

RESULTS

Plasma NO and Vascular responsiveness to NE, ACh and SNP: Vascular functions were impaired in the aortic rings of salt-loaded rats as evident by the decreased vascular relaxation response to ACh (P<0.05) (Figure 1) and SNP (P<0.05) (Figure 2), suppressed plasma NO (P<0.001) (Figure 4) and the increased vascular contractile response to NE (P<0.001) (Figure 3). Similarly, rat group combinedly fed a high salt diet and exposed to HET had diminished vascular functions, characterized by impaired vascular relaxation to Ach (P<0.001) and SNP (P<0.05), with a higher exaggerated contractile response to NE, but a paradoxically unaltered plasma NO (P>0.05) in comparison to control rat group. The exaggerated response to NE in the aortic rings of the experimental rats exposed to the combined factors possibly contributed to the severity of the observed salt-induced hypertension via an increased vascular tone. Interestingly, telmisartan, an ARB, normalized the impaired vascular function much more in the vessels of the rat group exposed to the combined factors by attenuating NE-mediated vascular contraction (P<0.001), while improving the impaired SNP-mediated vascular relaxation (P<0.05). Similarly, telmisartan mitigated the enhanced contractile response to NE in the vessels of the exclusively salt-loaded rats (P<0.001). Meanwhile, the vessels of rats exposed to H*Et al*one had impaired vascular response to Ach (P<0.01), with an associated diminished plasma NO (P<0.001) which were not improved by telmisartan (Table 1).

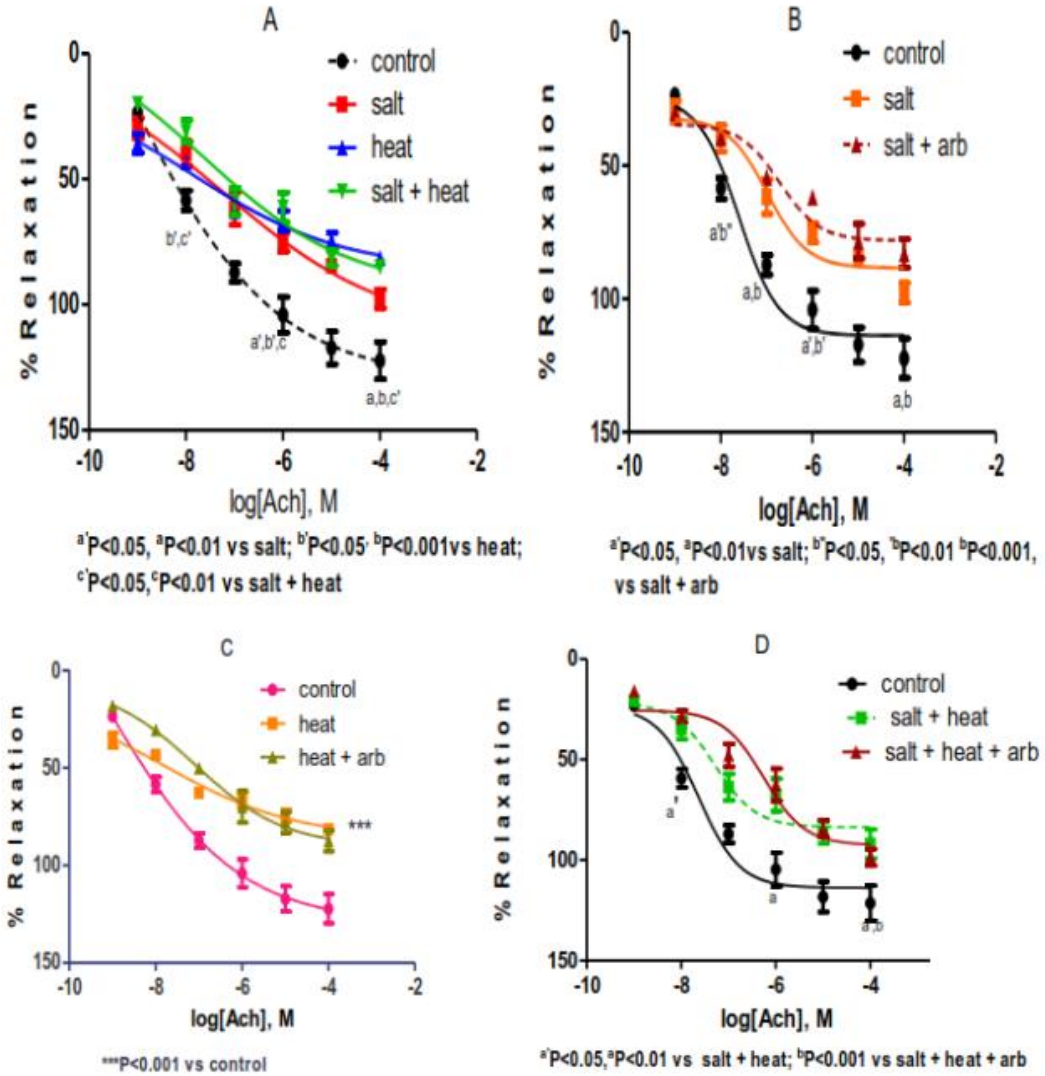

Figure 1:
Vascular relaxation responses to graded concentration of ACh (10-9 to 10-4 M) in precontracted aortic rings significantly impaired by high salt diet and high environmental temperature respectively and combinedly (figure A), with ARB (telmisartan 30mg/kg) showing no reversal influence on the impaired vascular responses (figure B, C, D).

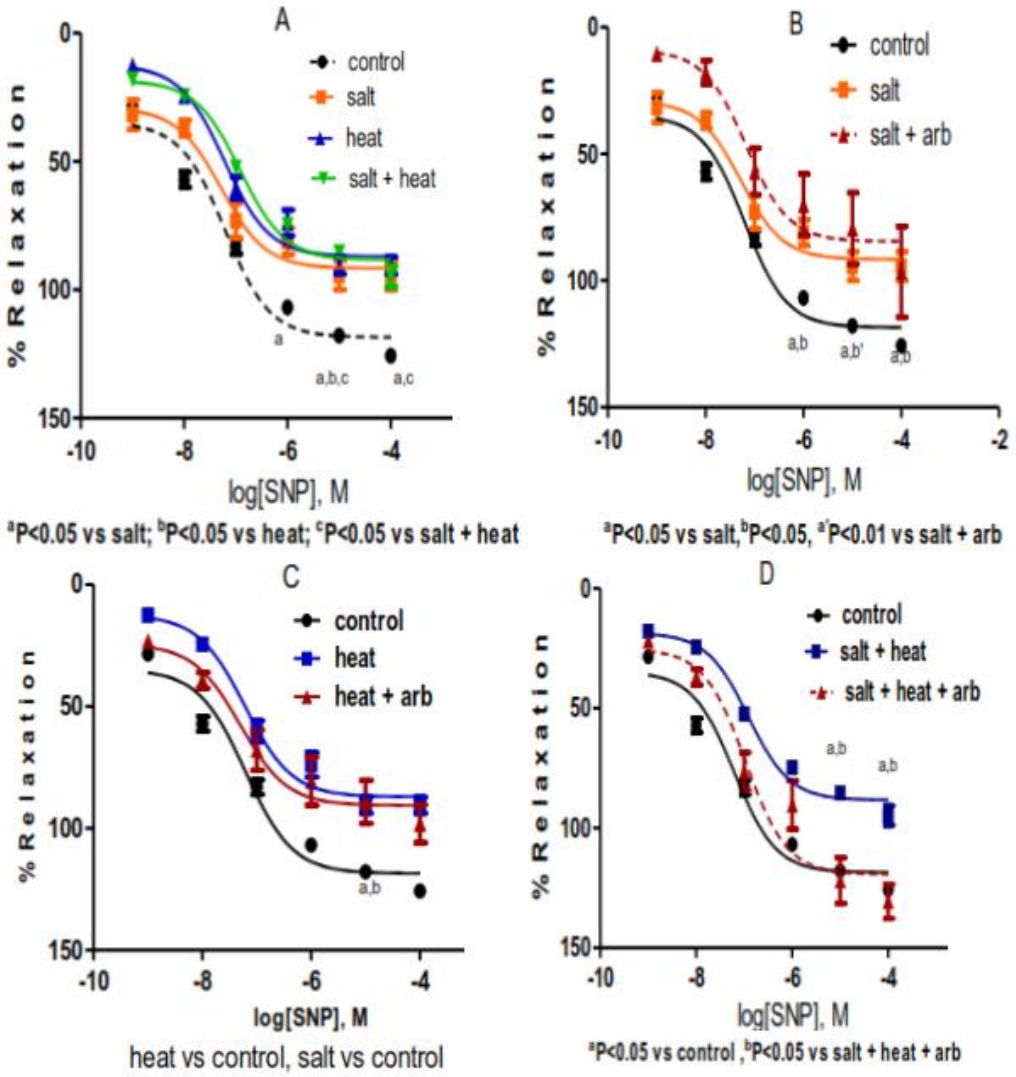

Figure 2:
Vascular relaxation responses to graded concentration of SNP (10^{-9} to 10^{-4} M) in precontracted aortic rings significantly impaired by high salt diet and high environmental temperature respectively and combinedly (figure A), with ARB (telmisartan 30mg/kg) showing reversal influence on the impaired vascular responses to the combined factors (figure D), but none to the individual factors (figure B and C).

Table 1:
Effect of HET on vascular responses to NE, ACh and SNP in aortic rings of salt-loaded rats with and without ARB

Groups	Maximum contractile Response to NE	$logEC_{50}$	Maximum relaxation to ACh	$logEC_{50}$	Maximum relaxation to SNP	$logEC_{50}$
control	53.0±4.1	-5.8±0.2	123.4±7.9	-4.5±2.9	125.8±1.8	-7.9±0.4
salt	93.8±14.6***	-7.2±5.4	92.1±4.9***	-8.0±5.8	92.8±4.8*	-7.3±0.2
heat	61.4±8.3	-6.6±0.3	81.5±1.9***	-6.0±1.9	104.5±5.2	-7.3±0.2
salt+heat	133.1±9.3***	-7.7±0.1	85.1±2.0***	-7.7±0.2	94.7±3.1*	-6.9±0.1
salt + arb	32.5± 3.4	-5.4±0.3	83.1±4.4***	-5.4±0.3	99.2±13.4	-7.2±1.0
heat + arb	76.1±2.6	-5.6 ± 0.1	87.6±5.4***	-5.6±0.1	99.9±6.4	-7.7±.1.0
salt + heat +arb	43.7± 4.7	-5.9± 0.3	98.7±4.1*	-5.9±0.3	130.6±6.0[†]	-7.0±0.2

Data expressed in % and presented as mean ± SEM. (n =6). **NE**:***P<0.001 vs control; **ACh**: *P<0.05,***P<0.001 vs control; **SNP**: *P<0.05 vs control, [#]P<0.05 vs heat; [†]P<0.05 vs salt + heat. arb: angiotensin II receptor blocker. ACh and SNP mediate endothelial dependent and independent relaxations respectively; NE mediates vascular contractions

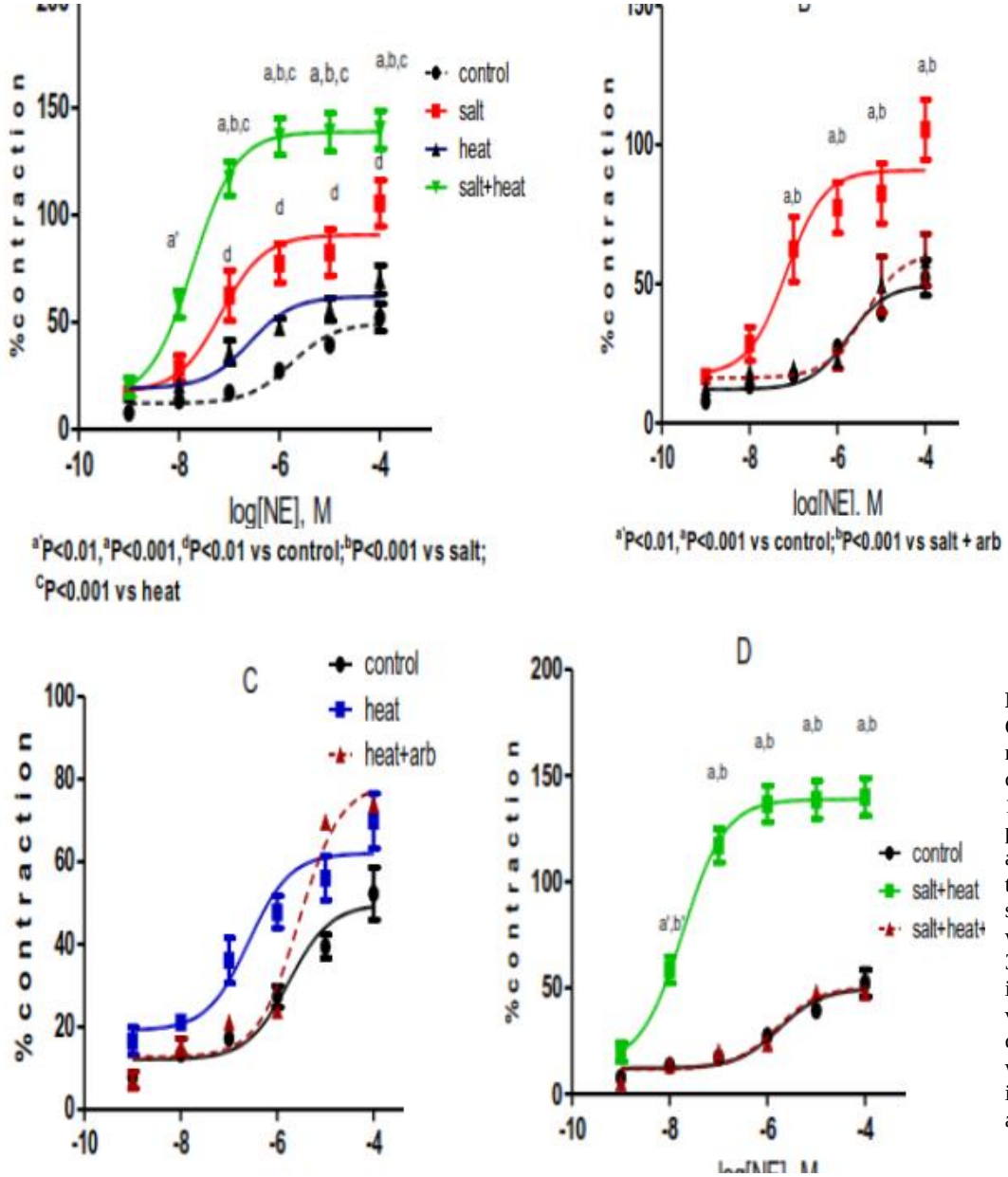

Figure 3:
Contractile vascular responses to graded concentration of NE (10-9 to 10-4 M) in aortic rings potentiated by high salt diet and high environmental temperature respectively and synergistically (figure A), with ARB (telmisartan 30mg/kg) showing reversal influence on the exaggerated vascular responses to the combined factors (figure D), with no change on the individual factors (figure B and C).

Figure 4:
Plasma Nitric Oxide level in salt-loaded rats exposed to environmental heat with and without ARB treatment.
****P<0.001 significantly lower compared with control;*
Data presented as Mean ± SEM (n= 6). ARB: angiotensin receptor blocker.

Vascular Photomicrography of abdominal aortic vessels of experimental rats: Transverse sections of abdominal aorta obtained from different experimental rat groups revealed vary degrees of structural changes compared to control rats. These changes were observed in rats fed a HSD and exposed to HET respectively and together. Specifically, these structural distortions include rough tunica intima characterized by intraluminal micropapillary projection of tunica intima and media, vary degrees of hypertrophy of tunica media characterized by increased collagen presence and inflammatory cell infiltrations. Collagen deposition and cellular infiltration features more prominently in the vessels of rat group exposed to H*Et al*one and combined with HSD. Most importantly, this vascular distortion extensively narrows the vascular lumen of the blood vessels of rat group exposed to the combined environmental factors. These histological distortions were noticeably mitigated by ARB in the rats exposed to combined environmental factors, but moderately in the rats fed either with HSD or exposed to HET (Figure 5)

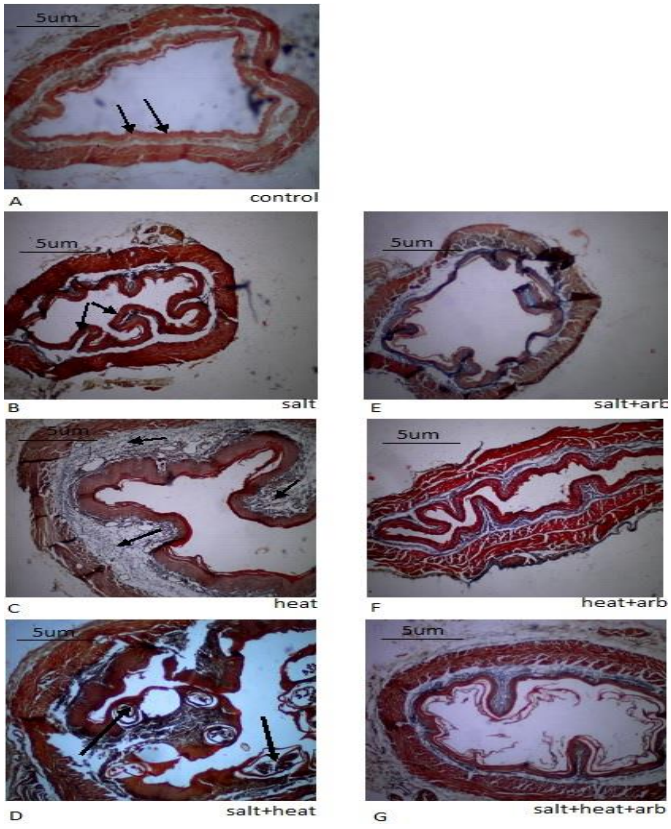

Plate 1:
Photomicrographs of abdominal aortic vessel in control rats (figure A) compared to rats fed with high salt diet (figure B),rats exposed to high environmental temperature (figure C) respectively and combinedly (figure D), with telmisartan, an ARB mitigating the deleterious vascular effect of the combined factors more (figure G) compared to its effect on the individual factors (E and F). Arrows pointing at the following features in section A= smooth and intact tunica intima; B = micropapillary projection of tunica intima and media, C = extensive collagen deposition, D = inflammatory cells infiltration; Magnification X40; Stained with masson's trichrome and counter stained with light green stain.

DISCUSSION

A balance between vascular endothelial relaxion and contraction is key to blood pressure regulation (Triggle *et al*., 2012). Hence, some vascular mechanisms implicated in the development of hypertension include hypertrophy of vascular smooth muscles, impairment of vascular and endothelial functions (Oloyo *et al*., 2016; Sofola *et al*., 2002), with associated reduction in the synthesis and availability of nitric oxide (Hermann *et al*., 2006). In this present study, vascular contractility response to norepinephrine (NE) was potentiated in the aortic rings of experimental rats fed a high salt diet in line with previous studies (Adegunloye & Sofola, 1997; Sofola *et al*., 2002). Similarly, potentiated vascular contractile responses to NE were also observed in the vessels of rats exposed to heat stress in agreement with the study by Geng *et al*. (2016), but contrary to the work of Massett *et al*. (1998), that reported non-significant increases in vascular response to NE.

Interestingly, aortic vessels from rats combinedly fed a high salt diet and exposed to hot environment exhibited incredibly exaggerated responses to NE compared to aortic vessels obtained from rats independently fed on high salt diet or exposed to high environmental temperature. This synergistic vascular contractile response to NE was nonetheless normalized in the vessels of rats treated with telmisartan under the combined environmental factors. Similarly, telmisartan blunted the expected vascular response to NE in the rat group exclusively fed a high salt diet. This is intriguing as the modulatory effect of telmisartan on vascular response to NE translated into blood pressure reduction in the group of rats jointly exposed to high salt diet and hot environment, which in contrast was not the case in the rat group exclusively fed on high salt diet in our earlier study (Agbaraolorunpo *et al*., 2019). Likewise, the enhanced vascular response to NE in the vessels of rats exclusively exposed to environmental heat, was not corrected by telmisartan. Apparently, the synergistic effect of high salt diet and environmental heat on vascular response to NE, normalized by telmisartan, potentially contributed to the observed exaggerated blood pressure rise and its subsequent attenuation by telmisartan in our earlier study (Agbaraolorunpo *et al*., 2019). This current finding is in conformity with a study that showed that losartan, a blocker of AT1 receptor, blunted salt-enhanced vascular reactivity to phenylephrine (Susic *et al*., 2010). As revealed by our current result, high environmental temperature appears to sensitize angiotensin type 1 receptors response to high salt activity for better ARB inhibitory effect. This is indeed instructive given the suggestion that blockade of angiotensin II type-1 receptor increases salt sensitivity in Sprague-Dawley rats (Endo *et al*., 2009).

Furthermore, vascular relaxation response to acetylcholine (ACh) was observed to be impaired in the vessels of rats exposed to high environmental temperature alone, in line with the work of Massett *et al*. (1998). This was ditto for vessels of the rats fed a high salt di*et al*one as indicated in earlier similar work (Barić *et al*., 2019). Likewise, the vessels of rats exposed to these combined factors showed similar impaired vascular relaxation to ACh with no additive effect. These observations suggest that high salt diet and environmental heat respectively impaired endothelial dependent relaxation, with no additive effect. One plausible explanation for this is that the contribution of nitric oxide (NO) to endothelium-dependent relaxation wane in response high salt diet (Nurkiewicz *et al*., 2010; Raffai *et al*., 2011) . This is thought is supported by studies which averred that chronic exposure to cardiovascular risk factors such as high salt diets, hypertension, hyperglycaemia, hyperlipidaemia, hyperhomocysteinemia suppress the production of NO from endothelium, while inducing a disturbance in the balance between endothelial relaxant and contractile factors (Mudau *et al*., 2012; Radenković *et al*., 2013). Again, patients with essential hypertension have been shown to have impaired basal nitric oxide release (Ghiadoni *et al*., 2000). Meanwhile, the rats treated with telmisartan under the aforementioned environmental conditions had no improvement in their vascular relaxation response to ACh, suggesting that telmisartan, an angiotensin receptor blocker, did not improve

the impaired endothelial dependent vascular relaxation in this study.

The current study also showed that the maximum vascular relaxation to SNP, a NO donor, was comparably impaired in the blood vessels of rat group fed a high salt di*et al*one and combined with environmental heat. This is in agreement with previous study which showed that high salt intake directly affected vascular smooth muscle via the impairment of vascular relaxation response to NO (Kagota *et al*., 2002). According to the study, vaso-relaxation via cGMP, excessive dietary salt causes the down-regulation of soluble guanylate cyclase, consequently diminishing cGMP production required in vascular smooth muscle relaxation.

Interestingly, telmisartan mitigated the impaired SNP-mediated vaso-relaxation in the blood vessels of the rats exposed combinedly to high environmental temperature and fed a high salt diet, with no effect in the vessels of rats exposed to either of the environmental factors. Evidently, the enhanced vascular response to NE and the accompanying impaired endothelial dependent and independent relaxation responses to ACh and SNP respectively may provide insight accounting for the elevated blood pressure pictures in our previous work (Agbaraolorunpo *et al*., 2019). Furthermore, the result of this current study supports the potential role of ARB in the attenuation of blood pressure via the suppression of vascular response to vasoactive agent like NE and reciprocal improvement of endothelial independent mechanisms, especially under the combined environmental factors.

Meanwhile, we observed that plasma NO level was significantly suppressed in all the experimental rat groups, including all groups treated with telmisartan compared with control rats. This is in harmony with the observed impaired ACh-mediated endothelial dependent vasorelaxation in this study. However, the level of NO in rats exposed to the combine environmental factors was observed to be paradoxically maintained similar to the level observed in the control rats. Although this was also expected to align with the observed endothelial dependent vaso-relaxation in the vessels of the rats exposed to the dual environmental assaults, this was not the case. This could be due to a compensatory response attempting to counter the excessive blood pressure rise in this rat group. This thought is in sync with earlier work which suggest that enhancement of NO production in experimental and genetic hypertensive rats operate as compensatory mechanism for vascular relaxation (Chen *et al*., 1997). Furthermore, this observation suggests that ARB-mediated blood pressure attenuation in this current study may be independent of circulatory NO modulation.

In tandem with the above observation, results from this present study also showed photomicrography evidence suggestive of vascular remodeling in the abdominal aortic vessels obtained from rats fed a high salt alone and those exposed to environmental heat respectively or combined. These changes were moderately attenuated in the rats treated with telmisartan under these respective environmental conditions. This is indeed important as Ang II has been demonstrated to act via AT1 receptors to promotes proliferation factors, vascular growth and hypertrophy (Esper *et al*., 2006), with the works of Lassègue *et al*. (2001) and Wang *et al*. (2001) providing the molecular basis for the effects of Ang II on vascular smooth muscle cell growth via proteins Akt and p38 mitogen-activated protein kinase signals. Meanwhile, the vascular histological manifestation in support of vascular remodeling in this present study include micropapillary projection of tunica intima and media, increased collagen in media wall, irregularities in the lining of intima and hypertrophy of vascular wall characterized by increased wall thickness. Evidently, these histological changes were more prominent and gross in the vessel of rats exposed to the combine factors compared with the rats exposed to either of the environmental factors. Meanwhile, these changes were better mitigated by telmisartan in the blood vessels of the rats exposed to the combined environmental factors than in the vessels of rats exposed to the individual factors. This is in tandem with the effective improvement of non-endothelial dependent vascular relaxation in the vessels of the rats exposed to the combined factors with ARB (angiotensin II receptor blocker) treatment in this current study. This possibly contributed to the blood pressure reduction under the combine influence of HSD and HET as we previously observed (Massett *et al*., 1998). The aforementioned observation partly aligns with the previous finding that Angiotensin II receptor blocker, telmisartan attenuated aortic stiffening and remodeling in STZ-diabetic rats (Salum *et al*., 2014).

In conclusion, chronic exposure to high environmental temperature exaggerated vascular contractile responses to high salt diet and vascular remodelling in rat model. However, telmisartan, an angiotensin II receptor inhibitor mitigated these changes and reversed the impaired vascular endothelial independent responses to the combined factors, an observation supporting a plausible role for angiotensin II type1 receptor in this vascular mechanism. This mechanism plausibly contributed to the earlier observed worsen severity of hypertension engender by a combination of high salt diet and chronic exposure to high environmental temperature. Angiotensin II receptor blocker monotherapy may therefore be promising in individual exposed to these dual environmental factor, characteristics of the black population in the sub-Saharan Africa.

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