Effect of Adrenomedullin on Cardiovascular Response to Sympathetic Autonomic Stress in Normotensive and Hypertensive Nigerians

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Abstract—Adrenomedullin is a potent vasodilator with established cardio-protective potentials when the body is exposed to stress. Sympathetic autonomic stress can lead to systolic (SBP) and diastolic blood pressure (DBP) reactivity which is a predictor of stroke. This study was to determine the effect of Adrenomedullin on cardiovascular response to sympathetic autonomic stress using the Cold Pressor Test in normotensive (NT) and hypertensive (HT) adults. Cardiovascular response to the CPT was measured among 30 NT and 30 HT participants, via blood pressure and heart rate before and after exposure to the cold pressor test (CPT). Plasma Adrenomedullin (ADM) was also determined by means of ELISA. Data were expressed as mean±S.E.M and statistical significance was accepted at 95% confidence interval. Exposure to CPT led to significant increases in SBP and DBP in both NT and HT participants with greater effect in HT (p<0.0001). Plasma Adrenomedullin was significantly higher among the NT but the difference on effect of response to CPT in both groups of participants was not significant. There was a negative and insignificant correlation between the ADM level and change in SBP and DBP among the NT participants but a positive and insignificant correlation with both SBP and DBP among the HT participants. We conclude that plasma Adrenomedullin may prevent the development of hypertension in black individuals.

Index Terms—Adrenomedullin; Autonomic Stress; Cold Pressor Test; Vascular reactivity; hypertension.

I. INTRODUCTION

Stress is defined as a subjective perception of an adverse environmental change which leads to a stress response allowing for adaptation to the new condition [1]. Stressors can either be physical or psychological in nature, warranting the stimulation of separate regions and complex pathways of the nervous system, causing them to elicit appropriate varied responses [2]. It is also becoming increasingly clear that the sympathetic-medullo-adrenal axes seem to be important in the response to stress. Stress could be acute or chronic, where acute stress occurs at all once and chronic stress happens over a long period of time [3]. The common types of stress include family and marriage difficulties, financial problems, job stress, physical or mental illnesses, school stress, substance abuse, loneliness, caring for the aged [3].

Blood pressure and heart rate have been known to be increased by chronic stress which makes the heart work very hard to be able to meet the metabolic needs of the body [3]. Stimulation of various regions of the cerebellum causes changes in arterial heart rate and blood pressure [4], [5]. Long term blood pressure elevation as seen in essential hypertension can lead to myocardial infarction, abnormal heart rhythm, heart failure and stroke [3].

Adrenomedullin (ADM) is a very important multifunctional peptide hormone involved in cardiovascular regulation. It is found in the adrenal medulla of most mammals, including humans [6]. It has also been found in the different parts of the circulatory system including the atria, ventricles and blood vessels, as well as cardiovascular, renal, pulmonary, cerebrovascular, gastrointestinal, endocrine tissues [7]. According to [8], ADM is actively involved in the mechanism responsible for protecting blood vessels from damage and remodeling thereby decelerating the process of ischemia in tissues and organs. Vascular dysfunction is affected by an imbalance in humoral and mechanical factors that have the innate function of modulating vascular functions thus leading to alteration in vascular integrity and modulation of lipid and glucose metabolism [9]. Adrenomedullin has been shown to activate an endothelium-dependent vasodilatation using the NO-cyclic GMP (cGMP) pathway in the aorta of rats, vascular bed of rat and canine kidneys [10]. In man, this peptide has been reported to be a potent vasorelaxant as well as a natriuretic peptide [11], [12].

Temperature and other environmental stressors are known to affect blood pressure and heart rate. The changes observed following the cold pressor test (CPT) are directly related to vascular response and pulse excitability [13]. The CPT is a classic stress induction task used in a wide range of research aimed at examining the relationship between stress, cognition, emotion and behavior [13]. Exposure to an extreme environment such as heat and cold is a form of stress experienced by all organisms [14]. In our earlier report, both normotensive and hypertensive participants had shown some tendency towards hyperreactivity of blood pressure in response to the CPT [15]. The effect of stress on daily living cannot be overemphasized as it is experienced by the average human being causing a variety of effects that may eventually lead to or worsen hypertension. The adverse effect of stress is felt to varying degrees depending on the inherent health status of individuals. As ADM has been suggested to play a protective role against acute stress [16], we sought to evaluate its effect on the cardiovascular response to sympathetic autonomic stress.
II. MATERIALS AND METHOD

Thirty normotensive (NT) and thirty hypertensive (HT) adults took part in the study after informed consent was obtained from them. Questionnaires were administered to all participants where relevant history was obtained. Ethical approval for this study (LREC. 06/10/992) was obtained from the Health Research and Ethics Committee of the Lagos State University Teaching Hospital, Ikeja. The experiments were carried out in accordance with Helsinki Declaration [17] in the Research Laboratory of the Department of Physiology, Lagos State University College of Medicine, Nigeria.

A. Inclusion Criteria

Participants were included if they were normotensive with blood pressure <140/90 mmHg or hypertensive with blood pressure ≥ 140/90 mmHg [18].

B. Exclusion Criteria

Following a general health check, volunteers were excluded if they had any history of smoking, if they had pulmonary, cardiovascular or metabolic diseases or if they were on treatment for such.

C. Procedure

Participants were advised to refrain from exercise at least 3 hours before the experiments and to avoid caffeine-containing beverages on the morning of the tests. Upon arrival at the laboratory, participants were allowed to rest for 10 minutes before the commencement of the procedure. The experiments were carried out in two sessions:

During the first session, weight (kg), height (m), blood pressure (mmHg) and heart rate were measured. The second session comprised of exposure to cold pressor test (CPT) followed by venesection to obtain blood sample for plasma Adrenomedullin assay. The CPT was carried out by asking the participants to insert their left foot in ice water maintained at 4°C, for 1 minute [15], [19]. The room temperature was maintained at 27°C by means of an air-conditioner system throughout the experiments.

D. Determination of Plasma Adrenomedullin

Plasma Adrenomedullin was measured with a 2-CAT ELISA- Fast Track (Rocky Mountain Diagnostics Inc., Colorado Springs, Colorado). The Adrenomedullin kit was purchased directly from the manufacturer (SunLong Bio-tech Co Limited, China).

Subject’s left forearm was wiped with alcohol swab and a tourniquet was tied 4cm above the cubital fossa to make the cubital vein more prominent. About 3mls of blood was withdrawn into an EDTA bottle from the cubital vein. The blood sample was promptly separated following centrifugation at an angular speed of 3000 revolutions per minute for 20 minutes. The plasma was then separated from each sample using a serological micropipette into 2ml cryovial tubes. These plasma samples were immediately stored in a minus 80 °C freezer until analysed for Adrenomedullin at a later date with Enzyme-Linked Immunosorbent Assay (ELISA) which is a plated-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies and hormones. The ELISA procedure was carried out according to product instructions. Briefly, wells were set for standard in a micro Elisa strip plate. Samples were loaded into the bottom of each well without touching the well wall and mixed well with gentle shaking. Samples were then incubated for 30 minutes at 37 °C after being sealed with closure plate membrane. Concentrated washing buffer was diluted with distilled water 30 times. Closure plate was peeled off afterwards and washed using the wash solution for 5 times. Then 50µl HRP-Conjugate reagent was added to each well except the blank control well and incubation followed by washing were repeated as described earlier. 50µ Chromogen solution A and 50µ Chromogen solution B were added to each well and mixed with gentle shaking and was incubated at 37 °C for 15 minutes. Light was avoided during colouring. 50µ stop solution was then added to each well to terminate the reaction. The colour in the well changed from blue to yellow. Absorbance was read at 450nm using a microtiter plate reader. Optical density value of the blank control well was set at zero. Assay was carried out 15 minutes after adding stop solution.

E. Data Analysis

Pulse Pressure was taken as the difference between the systolic and diastolic blood pressure. Mean Arterial Blood Pressure (mmHg) was calculated as diastolic blood pressure plus one-third of pulse pressure. Body Mass Index (kg/m2) was calculated using Quetelet’s equation which is Weight in kilograms divided by the square of the height in square metres [20]. Vascular Reactivity was determined as the change in systolic or diastolic blood pressure following the CPT. Values ≥15 mmHg were taken to be hyperreactive while normoreactive participants were those with a blood pressure (systolic or diastolic) change of ≤15 mmHg [15], [21]. Magnitude of response was taken as the percentage difference (%Δ) calculated as the difference in systolic and diastolic blood pressure before and after CPT, divided by level before CPT, multiplied by 100 [15].

F. Statistical Analysis

This was carried out using GraphPad Statistical Software, Prism 5 for Windows (GraphPad Software, San Diego, California, USA). The data were expressed as X ± standard error of the mean. Student paired and unpaired t-test were used to determine the difference in cardiovascular response to CPT. Correlation coefficients (r values) between the different studied parameters were determined using Spearman’s rank correlation. Statistical significance was accepted at 95% confidence interval.

III. RESULT

The normotensive participants were significantly younger than (p < 0.001) the hypertensive group. Other biodata of the participants were as shown in Table I.

A. Baseline Cardiovascular Parameters of Participants

Systolic blood pressure among the NT participants was 114 ± 1 mmHg which was significantly lower (p <0.0001) than the 140 ± 3 mmHg of the HT participants (Fig. 1). Also, diastolic blood pressure for the NT participants was 76
± 1 mmHg which was significantly lower (p<0.001) than the 84 ± 2 mmHg among HT participants (Fig. 1). As shown on the same Fig., the same trend was observed in Pulse Pressure (38 ± 1 mmHg NT vs 56 ± 3 mmHg HT) and Mean Arterial Blood Pressure (89±1 mmHg NT vs 103±2 mmHg HT) (p<0.001). The mean heart rate for the NT participants was 76±2 bpm and was not significantly different (p>0.05) from that of the HT participants which was 80±2 bpm (Fig. 2).

B. Effect of Cold Pressor Test on Blood Pressure

As shown in Fig. 2a, following exposure to the CPT, systolic blood pressure increased significantly (p<0.0001) among the two groups from the baseline of 114±1 mmHg to 136±2 NT and from the baseline of 141±3.4 mmHg to 165±4 mmHg HT. Among the NT participants, the diastolic blood pressure increased significantly (p<0.0001) from a baseline of 76±1 mmHg to 90±1 mmHg after exposure to the CPT (Fig. 2a) while that of the HT participants also significantly (p<0.0001) rose from a baseline of 84±2 mmHg to 98±2 mmHg following exposure to CPT (Fig. 2b). Similarly, pulse pressure increased significantly (p<0.0001) in both group of participants from a baseline of 38±2 mmHg to 46±2 mmHg amongst the NT participants (Fig. 2a) and from the baseline of 56±3 mmHg to 67±3 mmHg among the HT participants (Fig. 2b). Also, mean arterial blood pressure increased significantly (p<0.0001) amongst both the NT and HT participants from a baseline of 89±1 mmHg to 105±1 mmHg amongst the NT as shown in Fig. 2a and from a baseline of 103±2 mmHg to 120±3 mmHg amongst HT participants (Fig. 2b).

C. Effect of the Cold Pressor Test on Heart rate

When the participants were exposed to CPT, heart rate among the NT participants rose significantly (p<0.0001) from 76.4±1.6 bpm to 91.3±1.7 bpm and from 80.6±1.9 bpm to 95.4±1.7 bpm amongst the HT participants (p<0.0001) as shown in Fig. 3.

D. Vascular Hyperreactivity and Magnitude of Response to the Cold Pressor Test

Systolic hyperreactivity was 83% among the NT participants which was slightly higher than (p=0.17) the 77% among the HT participants. Diastolic hyperreactivity was also not significantly different (p=0.85) between the normotensive and hypertensive participants at 40% and 47% respectively as shown in Table II. Similarly, as shown on the same Table, the response of the participants (depicted by the magnitude of change) to the CPT was not significantly affected by their blood pressure status.

Table I: Biodata of Participants

|                     | Normotensive (n=30) | Hypertensive (n=30) | p       |
|---------------------|---------------------|---------------------|---------|
| Age (year)          | 36.77 ± 1.77        | 58.33 ± 1.85        | <0.0001 |
| W (kg)              | 71.58 ±2.07         | 77.30 ± 2.07        | >0.05   |
| H (m)               | 1.63 ± 0.02         | 1.62 ±0.02          | >0.05   |
| BMI (kg/m²)         | 27.18 ± 0.77        | 29.71 ± 0.94        | <0.05   |

Key: W: Weight; H: Height; BMI: Body Mass Index
Fig. 3: Heart rate response of the normotensive and hypertensive participants to Cold Pressor Test

**KEY:** *** = p<0.0001 heart rate before CPT vs after CPT

CPT = cold pressor test; Beats/min = beats per minute

Table II: Systolic and diastolic hyper-reactivity amongst the normotensives and hypertensive participants

|                   | Normotensive (n=30) | Hypertensive (n=30) | p     |
|-------------------|---------------------|---------------------|-------|
| SHR (Δ%)          | 83% (21.5±1.6)      | 77% (24.5±20)       | 0.2   |
| DHR (Δ%)          | 40% (13.1±1.0)      | 47% (13.4±1.5)      | 0.8   |

**KEY:** SHR: Systolic Hyperreactivity; DHR: Diastolic Hyperreactivity; Δ% = Magnitude of Response

Fig. 4: Adrenomedullin values of normotensive and hypertensive participants

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**Fig. 5:** Correlation between the change in systolic (a) and diastolic (b) blood pressure and plasma Adrenomedullin among normotensive participants

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**KEY:** SHR: Systolic Hyperreactivity; DHR: Diastolic Hyperreactivity; Δ% = Magnitude of Response

**E. Plasma Adrenomedullin Levels**

Adrenomedullin level amongst the NT participants was 1.4±0.3 ng/L and was only slightly higher than the 0.33±0.11ng/L among HT participants (p = 0.065) as shown in Fig. 4. There was a negative but statistically nonsignificant correlation between the Adrenomedullin (ADM) level and change in SBP (r = -0.11; p = 0.5) and DBP (r = -0.07; p = 0.7) among the NT participants while among the HT participants, ADM was positively and statistically nonsignificantly correlated with SBP (r = 0.04; p = 0.98) and DBP (r = 0.20; p = 0.28). These were shown in Fig. 5(a)(b) and Fig. 6(a)(b).
Exposure to the CPT has the ability to cause an increase in physiological response in a controlled environment. Some previous studies [15], [23], [27] have reported the hypertensive cohort of participants responding with greater hyperreactivity than the normotensive participants [15]. This could be due to the significant difference in the age of the two groups in the present study as a result of which the blood vessels among the younger normotensive participants was still more responsive to the cold stress. However, in a slightly contradicting report by [23], the hypertensive participants in their study experienced significant increases in blood pressure and heart rate following exposure to the CPT whereas heart rate did not seem to be significantly affected among the normotensive participants. Increase in heart rate is likely due to a massive release of catecholamines from the sympathetic nervous system caused by exposure to the cold pressor test. This activates the β adrenergic receptors in the sinoatrial node of the heart leading to an increase in heart rate [24]. In the present study, heart rate was observed to increase in both normotensive and hypertensive participants, though more remarkably in the latter group. This is indicative of a sustained autonomic activation [25], [26].

Findings from this study demonstrate systolic and diastolic hyperreactivity amongst the participants on exposure to CPT where systolic hyperactivity was more prominent. This is in keeping with the findings from [27] where it was established that temperature changes had a more prominent effect on systolic blood pressure than diastolic blood pressure. This result also shows clear similarity with our earlier study [15] though systolic hyperreactivity in response to the CPT seems to be more elaborate in the present study. The assessment of hyperactivity to cold pressor test in both cohort of participants is important because systolic blood pressure (SBP) reactivity is associated with increased risk of cerebrovascular accident and this relationship might be a reflection of an acute increase in cardiac force during systole [28]. The higher the reactivity, the higher the risk of cardiovascular events such as stroke and myocardial infarction [28]. According to [29], blacks have a higher risk of vascular reactivity to stress than any other race. This is very well demonstrated in the hyperreactivity findings in this study which is above 70% amongst both the normotensive and hypertensive cohorts.

That the magnitude of systolic blood pressure response to CPT is higher than that of diastolic blood pressure in both the normotensive and hypertensive participants is in keeping with the report by [30]. This may be due to increased cardiac contractility brought about by sympathetic innervation thus influencing systolic blood pressure. One significant finding that our study brought forth is that the normotensive participants had a higher magnitude of both systolic and diastolic responses contrary to the findings in our earlier study [15]. There is a possibility of some form of cardiovascular compensation among the hypertensive participants which may have been responsible for the slightly lower magnitude of systolic and diastolic responses.

Contrary to previous reports such as those of [31] and [32], the plasma level of Adrenomedullin (ADM) was higher in the normotensive participants than in the hypertensive participants in this study. This may be the reason the normotensive participants still had their blood pressure level within the normal range as the high level of systolic and diastolic responses in both the normotensive and hypertensive participants is in keeping with the findings in this study which is above 70% amongst both the normotensive and hypertensive cohorts.

IV. DISCUSSION

The present study showed a significant increase in systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate following exposure to the cold pressor test (CPT). Our results are in keeping with the findings of some previous studies [15], [22]. The CPT was used to stimulate, ethically, a stressful situation in order to induce a physiological response in a controlled environment. Exposure to the CPT has the ability to cause an increase in blood pressure. Whereas this increase was observed more among the normotensive participants in this study, earlier reports have recorded the hypertensive cohort of participants responding with greater hyperreactivity than the normotensive participants [15]. This could be due to the significant difference in the age of the two groups in the present study as a result of which the blood vessels among the younger normotensive participants was still more responsive to the cold stress. However, in a slightly contradicting report by [23], the hypertensive participants in their study experienced significant increases in blood pressure and heart rate following exposure to the CPT whereas heart rate did not seem to be significantly affected among the normotensive participants. Increase in heart rate is likely due to a massive release of catecholamines from the sympathetic nervous system caused by exposure to the cold pressor test. This activates the β adrenergic receptors in the sinoatrial node of the heart leading to an increase in heart rate [24]. In the present study, heart rate was observed to increase in both normotensive and hypertensive participants, though more remarkably in the latter group. This is indicative of a sustained autonomic activation [25], [26].

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ADM exerts a vasodilatory effect thus protecting the participants from the development of high blood pressure. This is in contrast to the findings by [33] who reported that Adrenomedullin acts centrally to raise systemic blood pressure. However, correlating ADM levels with the systolic and diastolic blood pressure responses to CPT showed an insignificant negative correlation amongst the normotensives and a positive correlation among the hypertensives. This could be a pointer to the different mechanisms of action relevant to the blood pressure status of the individuals. Adrenomedullin is expected to modulate the response to stress. It is directly implicated in countering further increase in blood pressure as a result of its biological role in the protective and compensatory mechanism in the cardiovascular system [34]. Also, [35] reported that cerebellar ADM plays an important role in the regulation of blood pressure via dysregulation of cerebellar ADM-system during hypertension. However, further studies are required to ascertain the exact role of ADM in response to CPT.

V. CONCLUSION

We conclude that sympathetic autonomic stress can lead to systolic and diastolic blood pressure hyperreactivity which is a predictor of cardiovascular accidents. Plasma Adrenomedullin may prevent the incidence of high blood pressure in normotensive participants but may have a different mechanism of action among hypertensive individuals. It is also possible that the peptide hormone contributed to the greater reactivity among the normotensive subjects. Going forward, more mechanistic research will be carried out to compare ADM action among hypertensive individuals who are on treatment and those who are treatment–naïve.

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