Protocol

Title: The Role of Aldosterone on Exercise Pressor Reflex in Hypertension , NCT01996449
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Investigators

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1. Introduction and Purpose:

Hypertensive patients often show an exaggerated rise in blood pressure (BP) during exercise—sometimes to the levels that raise safety concern. The mechanisms responsible for exercise pressor reflex (EPR) overactivity in hypertension remain unknown but an increasing body of evidence suggests a role for aldosterone in regulating resting central sympathetic outflow in both hypertensive rats and humans. Furthermore, local aldosterone synthesis in the brain has been shown to contribute to sympathetic activation and hypertension in SHR and Dahl-salt sensitive rats despite normal circulating levels of aldosterone. Whether aldosterone contributes to excessive sympathoexcitation and BP elevation during exercise remains to be investigated. Therefore, we now propose parallel translational studies in hypertensive patients with primary hypertension and a secondary form of hypertension caused by adrenal overproduction of aldosterone (primary aldosteronism) to systematically investigate the contribution of aldosterone to the generation of EPR dysfunction.

Major New Hypothesis: Aldosterone augments the EPR by selectively potentiating muscle mechanoreflex activity in hypertensive humans. We further hypothesize that this effect of aldosterone is mediated by its direct action on the central nervous system and that mineralocorticoid receptor (MR) antagonists constitute an effective treatment for EPR overactivity in hypertension, independent of reductions in resting BP.

In patients with uncomplicated essential hypertension (EH), hypertensive patients with primary aldosteronism (PA), and normotensive (NT) controls matched for age, gender, ethnicity, and BMI, we will perform multiple exercise interventions to determine if:

a. Sympathetic nerve activity (SNA) and BP responses to EPR activation during rhythmic handgrip exercise and mechanoreflex activation during passive arm cycling are augmented in patients with EH and PA.
b. SNA and BP responses to metaboreflex activation during post handgrip arm occlusion are unaltered in the EH and PA groups.
c. Indices of central command (increases in skin SNA, heart rate, and rating of perceived effort) input during exercise are similarly increased in EH and PA groups compared to the NT group. In addition, the sensitivity of the arterial baroreflex to modulate SNA and BP responses to exercise is unaltered during rhythmic and static handgrip in EH and PA groups.
d. Augmented EPR and mechanoreflex sensitivity in a subset of PA patients with aldosterone-producing adenoma (APA) is reversible upon surgical removal of the tumor.
e. MR antagonists constitute an effective treatment for EPR dysfunction by attenuating mechanoreflex overactivity in patients with EH and PA. Such effects have not been observed with treatment with amlodipine despite similar reductions in resting BP.

2. Background:

Hypertensive patients are known to display exaggerated rise in blood pressure (BP) during exercise but the underlying mechanisms are poorly understood. Normally, exercise is accompanied by decreased parasympathetic activity and increased sympathetic activity caused by central command and activation of thin fiber muscle afferents that reflexively increase sympathetic outflow and BP. Traditionally, muscle afferents were dichotomized as metaboreceptors, which are activated slowly and only during intense or ischemic muscle contraction, or mechanoreceptors, which respond quickly to even mild deformation of their receptive fields. The increase in SNA and BP caused by activation of these receptors, known as exercise pressor reflex, is normally buffered by activation arterial baroreceptors, which are reset to operate at higher BP range but at the same level of sensitivity. Our recent work in spontaneously hypertensive rats (SHR) and patients with essential hypertension indicated an exaggerated rise in sympathetic nerve activity (SNA) and BP during muscle contraction even in the absence of congestive heart failure. Furthermore, the latency of the sympathetic response during exercise is much shorter than normal and the threshold level of exercise intensity required to increase muscle SNA is much lower than normal, implicating mechanoreceptor mediation. Mechanisms responsible for overactive exercise pressor reflex (EPR) in hypertension remain unknown but an increasing body of evidence suggested a role for aldosterone in regulating resting central sympathetic outflow in both hypertensive rats and humans. Furthermore, local aldosterone synthesis in the brain has been shown to contribute to sympathetic activation and hypertension in the SHRs and Dahl-salt sensitive rats despite normal circulating levels of aldosterone. Whether aldosterone contributes to excessive sympathoexcitation and BP elevation during exercise remains unknown. Therefore, we now propose parallel translational studies in hypertensive patients with primary hypertension and secondary form of hypertension caused by adrenal overproduction of aldosterone (primary aldosteronism), using multiple interventions to directly activate muscle mechanoreceptors, metaboreceptors, or arterial baroreceptors during exercise and microelectrode recordings of SNA to measure the reflex response. The distinctive features of this proposal include: (1) the use of state-of- the-art techniques (microelectrode recordings of SNA) to directly test mechanistic hypotheses in conscious humans; (2) assessment of mechanoreflex using a new paradigm which is developed in our laboratory which evokes sustained elevation in SNA and BP than other methods previously described in humans; and (3) the use of complimentary approach to asses central vs. peripheral effects of aldosterone on neural control of circulation.

Significance and Cardiovascular Relevance: Hypertension is a major cause of cardiovascular morbidity and mortality in the United States and many countries worldwide. Excessive elevation in BP during exercise has been shown to contribute to impaired exercise tolerance in hypertensive patients even in the absence of coronary artery disease or left ventricular dysfunction [1-4]. Furthermore, numerous epidemiological studies indicated that BP during exercise predicts development of left ventricular hypertrophy [5, 6], stroke, myocardial infarction, and death [7-9], *independent of* resting BP. Accordingly, current guidelines *prohibit* any exercise in hypertensive patients if BP during exercise is 220/105 or above for safety reasons [10, 11]. Therefore, exercise prescription is often limited in hypertensive patients with an enhanced EPR [12]. Because most current antihypertensive drugs approved are much less effective in reducing BP at rest than during exercise [9, 13-18], a more complete mechanistic understanding of the augmented pressor response to exercise in human hypertension could lead to effective antihypertensive treatment. This will in turn allow more hypertensive patients to engage in regular exercise and enjoy the cardiovascular benefits of exercise, which is also an important nonpharmacologic treatment of hypertension. The information derived from our proposed studies will be pertinent not only to patients with primary hypertension but also those with primary aldosteronism which has been identified in 5-13% of unselected patients with hypertension in the community and primary care practice [19, 20] and up to 20% of patients with resistant hypertension referred to the tertiary care hypertension clinic [21].

3.Concise Summary of Project:

General Methods BP will be measured with an oscillometric device (Welch Allyn, N.C.).
(1) Microneurography. With the subject supine, we will measure blood pressure, heart rate, and muscle SNA with microelectrodes in the arm (median nerve or radial nerve or posterior cutaneous nerve) or leg (peroneal nerve). The nerve signals are amplified, filtered, rectified and integrated to obtain a mean voltage display of SNA. The criteria for an acceptable recording of muscle SNA has been previously described [15]. Sympathetic bursts will be detected by inspection of the filtered and mean voltage neurograms. A deflection on the mean voltage display is counted as a "burst" if it has a minimal signal to noise ratio of 2:1. SNA will recorded at rest in all protocols.

(2) Reflex Interventions: (a) Handgrip exercise . Rhythmic HG (RHG) will be performed in the nondominant arm at 40 beats per minute (50% cycle duty) at 30% and 45% maximal voluntary contraction (MVC), each for 3 minutes. Static HG (SHG) will be performed at 30% MVC for 2 minutes. Ratings of perceived exertion (RPE) will be obtained at the end of each exercise by using a 6- to 20-unit Borg scale. *For safety, the study will be terminated if BP during exercise at any time point is 220/105 mmHg or above in accordance with current guidelines [10, 11].* (b) Post-Handgrip Exercise Circulatory Arrest (PECA). During the last 5 sec of static HG exercise, a pneumatic cuff on the upper exercising arm will be inflated to 50 mmHg above systolic BP for 2 minutes to activate the metaboreflex. (c) Passive arm cycling. To activate mechanoreflex, the subject's nondominant hand will be strapped to one of the arm pedal of the arm ergometer while the investigator put their hand on the other pedal to move the arm ergometer at 80 r.p.m. Subjects will be asked to relax their arms during passive arm cycling for 5 minutes. Surface electromyography (EMG) on the bicep muscles (Noraxon, Scottsdale, AZ) will be used to monitor the muscular activity and a biofeedback device to assist the subjects with relaxation of their arm muscles. (d) Arterial baroreflex sensitivity. Arterial baroreflex control of muscle SNA and HR will be assessed during bolus injection of nitroprusside (NTP) and phenylephrine (PE) using the modified Oxford technique [22]. *For safety reasons, NTP will not be given if BP is ≤90/60 mmHg and PE will not be given if BP is ≥ 160/100 mmHg immediately prior to administration.*

(3) Cardiopulmonary testing: A subset of subjects who opt for this optional testing, will be asked to cycle on a rate-independent ergometer to measure peak VO2 using a metabolic cart (Medgraphics) during a separate visit. An established cycle ergometer ramp protocol will be performed. Standard indications for termination of exercise testing will be employed. Respiratory gases inclusive of VE, VO_2, VCO_2, and RER will be measured continuously throughout the exercise test. We will also measure continuous ECG and maximal heart rate, maximal oxygen uptake (peak VO_2), pulmonary ventilation (VE), ventilatory equivalents for oxygen (VE/VO_2), carbon dioxide (VE/CO_2), end-tidal partial pressure of oxygen ($PETO_2$), and carbon dioxide ($PETCO_2$). Simultaneous cardiac output (CO) measurement will be performed at rest and during steady state exercise testing by inert gas rebreathing method using Inncor system (Innovision A/S, Odense, Denmark). Cardiac output will be measured separately from maximal exercise testing in three conditions: (1) at rest, (2) at 20 Watts, and (3) at 50% peak VO2/work load. After the participant has completed the peak exercise testing protocol and rested 5 minutes, exercise stress echocardiography will be performed at 50% of maximal workload.

Premenopausal women will be studied during the early follicular phase (2-4 days after the onset of menstruation) to minimize confounding influence of sex hormones on SNA [23]. Women taking *oral contraceptives will be studied during low hormone (LH) phase (i.e., placebo pills).*

Agent/Device
A. Amlodipine
Trade name: Norvasc
Chemical Formula: 3-Ethyl-5-methyl (±)-2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-l,4-dihydro-6-methyl-3,5-pyridinedicarboxylate, monobenzenesulphonate

Source: Pfizer Inc.
Pharmacology: Inhibits calcium ion from entering the “slow channels” or select voltage-sensitive areas of vascular smooth muscle and myocardium during depolarization, producing a relaxation of coronary vascular smooth muscle and coronary vasodilation; increases myocardial oxygen delivery in patients with vasospastic angina.
Toxicity: Peripheral edema, fatigue, dizziness, hypotension, and gingival hyperplasia.

B. Eplerenone
Trade name: Inspra
Chemical Formula: Pregn-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3oxo-, γ-lactone, methyl ester, (7α,11α,17α)
Source: Pfizer Inc.
Pharmacology: Eplerenone binds to the mineralocorticoid receptor and blocks the binding of aldosterone, a component of the renin-angiotensin-aldosterone-system (RAAS). Aldosterone synthesis, which occurs primarily in the adrenal gland, is modulated by multiple factors, including angiotensin II and non-RAAS mediators such as adrenocorticotropic hormone (ACTH) and potassium. Aldosterone binds to mineralocorticoid receptors in both epithelial (e.g., kidney) and nonepithelial (e.g., heart, blood vessels, and brain) tissues and increases blood pressure through induction of sodium reabsorption and possibly other mechanisms.
Toxicity: hyperkalemia, orthostatic hypotension, and acute renal failure

4.Study Procedures:

Experiments will be performed on 3 groups of nondiabetic subjects 1) stage I (140-159/90-99 mmHg) subjects with EH, 2) stage I hypertensive subjects with PA, and 3) NT controls. The diagnosis of PA will be established based on the current Endocrine Society guidelines [24]. **As part as standard medical care**, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, renin inhibitors, central sympatholytic drugs and diuretics will be discontinued for at least 2 weeks and MR antagonists will be discontinued for at least 6 weeks to avoid interference with biochemical testing [24]. During the period of biochemical evaluation, subjects will be treated with calcium channel blockers, hydralazine, and/or alpha-adrenergic receptor blockers, which do not interfere with measurement of plasma renin activity (PRA) or aldosterone [24]. The screening evaluation is considered to be positive if plasma aldosterone levels are > 15 ng/dL and PRA are < 1 ng/ml/hr. Diagnosis of PA is established by presence of nonsuppressible aldosterone production after a salt loading test according to the guidelines [24]. Subjects with biochemical confirmation of PA will undergo adrenal vein sampling (AVS) **as part of standard care** to distinguish between unilateral vs. bilateral adrenal overproduction of aldosterone. We need to determine if association between aldosterone and SNA as well as BP is independent of dietary pattern, including caloric intake. Dietary pattern will be assessed by food recall using the Automated Self-Administered 24-Hour (ASA24®) Dietary Assessment Tool (https://epi.grants.cancer.gov/asa24/).

The following procedures are experimental:

We plan to assess muscle SNA and MAP at rest, during passive one-arm cycling exercise (see aim 2a), RHG, and SHG followed by PECA in 15 EH and 15 PA subjects (*not included in previous aims*). Subjects will be randomized to receive eplerenone (50-200 mg/day) or amlodipine (2.5-10 mg/day) each for 8 weeks, using randomized, double-blinded, crossover design. The rationale for using amlodipine is that previous studies have shown that this drug reduces BP without affecting resting SNA [26]. BP and serum K will be monitored every 2 weeks. Each subject will be started with 50 mg eplerenone/day or 2.5 mg amlodipine/day. If BP remains above 140/90 mmHg at week 2, the dose of eplerenone will be increased to 100 mg/day, whereas amlodipine will be increased to 5 mg/day. If BP remains elevated at week 6,

eplerenone will be increased to 200 mg/day and amlodipine to 10 mg daily. *This dose titration of amlodipine and eplerenone is chosen because it was shown to cause equivalent reductions in BP in hypertensive patients in a large clinical trial* [27]. If hyperkalemia develops with eplerenone, the dose will be reduced by 50% and serum K will be rechecked in 2 weeks (see detail in section 10d). We predict that eplerenone will attenuate the increase in SNA and MAP during HG and passive arm cycling in subjects with EH and PA without affecting SNA and BP responses to PECA. Amlodipine will have no effect on SNA or BP in the same subjects during all exercise interventions, despite causing similar reduction in resting BP.

Visit#1 (week 0): Obtain blood samples for basic metabolic panel, resting levels of plasma direct renin, aldosterone (aldo), norepinephrine (NE), and fasting plasma glucose and insulin. Then, assess muscle SNA and MAP at rest, during passive one-arm cycling exercise for 5 minutes (see general method #2c). During passive arm cycling, surface electromyography (EMG) on the bicep muscles (Noraxon, Scottsdale, AZ) will be used to monitor the muscular activity and a biofeedback device to assist the subjects with relaxation of their arm muscles. Then, subjects will be asked to perform, RHG at 30% and 45% MVC each for 3 minutes, and SHG at 30% MVC followed by PECA for 2 minutes. Each exercise intervention will be separated by at least 30 minutes. After completion of exercise, each subject will be randomized to receive Eplerenone at the dose of 50 mg once daily or Amlodipine 2.5 mg once daily.

Visit#2 (2 weeks later): Subjects will return for BP check and blood draw for basic metabolic panel. If BP is < 90/60 mmHg, subjects will be excluded from the study. If subjects BP > 140/90, the dose of Amlodipine will be increased to 5 mg once daily or Eplerenone will be increased to 100 mg once daily. 3cc of blood will be drawn for basic metabolic profile. If If hyperkalemia develops with eplerenone, the dose will be reduced by 50% and serum K will be rechecked in 2 weeks.

Visit#3 (2 weeks later): Subjects will return for BP check and blood draw. If BP is < 90/60 mmHg, the dose of study drugs will be reduced by 50%. If subjects BP > 140/90, the dose of Amlodipine will be increased to 7.5 mg once daily or Eplerenone will be increased to 150 mg once daily. 3cc of blood will be drawn for basic metabolic profile. If hyperkalemia develops with eplerenone, the dose will be reduced by 50% and serum K will be rechecked in 2 weeks.

Visit#4 (2 weeks later): Subjects will return for BP check and blood draw. If BP is < 90/60 mmHg, the dose of study drugs will be reduced by 50%. If subjects BP > 140/90, the dose of Amlodipine will be increased to 10 mg once daily or Eplerenone will be increased to 200 mg once daily. 3cc of blood will be drawn for basic metabolic profile. If hyperkalemia develops with eplerenone, the dose will be reduced by 50% and serum K will be rechecked in 2 weeks.

Visit#5 (2 weeks later): Subjects will return for blood draw for basic metabolic panel, resting levels of plasma direct renin, aldosterone (aldo), norepinephrine (NE), and fasting plasma glucose and insulin. Then, assess muscle SNA and MAP at rest, during passive one-arm cycling exercise for 5 minutes (see general method #2c). During passive arm cycling, surface electromyography (EMG) on the bicep muscles (Noraxon, Scottsdale, AZ) will be used to monitor the muscular activity and a biofeedback device to assist the subjects with relaxation of their arm muscles. Then, subjects will be asked to perform RHG at 30% and 45% MVC each for 3 minutes, and SHG at 30% MVC followed by PECA for 2 minutes. Then, subjects will be given BP medications in the remaining arm (either Amlodipine 2.5 mg once daily or Eplerenone at the dose of 50 mg once daily).

Visit#6 (2 weeks later): Subjects will return for BP check and blood draw. If BP is < 90/60 mmHg, subjects will be excluded from the study. If subjects BP > 140/90, the dose of Amlodipine will be increased to 5 mg once daily or Eplerenone will be increased to 100 mg once daily. 3cc of blood will be drawn for basic metabolic profile. If hyperkalemia develops with eplerenone, the dose will be reduced by 50% and serum K will be rechecked in 2 weeks.

Visit#7 (2 weeks later): Subjects will return for BP check and blood draw. If BP is < 90/60 mmHg, the dose of study drugs will be reduced by 50%. If subjects BP > 140/90, the dose of Amlodipine will be increased to 7.5 mg once daily or Eplerenone will be increased to 150 mg once daily. 3cc of blood will be drawn for basic metabolic profile. If hyperkalemia develops with eplerenone, the dose will be reduced by 50% and serum K will be rechecked in 2 weeks.

Visit#8 (2 weeks later): Subjects will return for BP check and blood draw. If BP is < 90/60 mmHg, the dose of study drugs will be reduced by 50%. If subjects BP > 140/90, the dose of Amlodipine will be increased to 10 mg once daily or Eplerenone will be increased to 200 mg once daily. 3cc of blood will be drawn for basic metabolic profile. If hyperkalemia develops with eplerenone, the dose will be reduced by 50% and serum K will be rechecked in 2 weeks.

Visit#9 (8 weeks later): Subjects will return for blood draw for basic metabolic panel, resting levels of plasma direct renin, aldosterone (aldo), norepinephrine (NE), and fasting plasma glucose and insulin. Then, assess muscle SNA and MAP at rest, during passive one-arm cycling exercise for 5 minutes (see general method #2c). During passive arm cycling, surface electromyography (EMG) on the bicep muscles (Noraxon, Scottsdale, AZ) will be used to monitor the muscular activity and a biofeedback device to assist the subjects with relaxation of their arm muscles. Then, subjects will be asked to perform RHG at 30% and 45% MVC each for 3 minutes, and SHG at 30% MVC followed by PECA for 2 minutes. End of study.

5. Inclusion Criteria

Experiments will be performed on 3 groups of nondiabetic human subjects 1) stage I (140-159/90-99 mmHg) subjects with essential hypertension, 2) stage I hypertensive subjects with primary aldosteronism, and 3) normotensive controls. Only subjects without CHF, any heart diseases, or target organ disease with eGFR ≥ 90 ml/min/1.73 m^2, can participate in the study.

6. Exclusion Criteria

(1) Any evidence of cardiopulmonary disease, left ventricular hypertrophy or systolic dysfunction by echocardiography.
(2) Blood pressure averaging ≥160/100 mmHg
(3) Estimated glomerular filtration rate (eGFR) < 90 mL/min/1.73m^2
(4) Diabetes mellitus or other systemic illness
(5) Pregnancy
(6) Hypersensitivity to nitroprusside, phenylephrine, amlodipine or eplerenone
(7) Any history of substance abuse or current cigarette use
(8) Any history of psychiatric illness
(9) History of malignancy

7. Sources of Materials:

The physician-investigators will have access to the patients' medical records. We also will have access to the Dallas Heart Study database, as all participants gave their written consent to be contracted for future research studies. The Dallas Heart Study health survey questionnaire data will be used to identify potentially eligible subjects to be recruited for our studies. However, all the new hemodynamic and hormonal data at rest and during handgrip exercise (SNA, BP, HR, plasma NE, insulin, glucose, serum K, renin, serum aldosterone, baroreflex sensitivity) will be obtained solely for research purposes.

8. **Recruitment Methods and Consenting Process:** Informed written consent will be obtained from all subjects using consent forms approved by the UT Southwestern IRB. The physician-investigators will interview all potential subjects and will explain the nature of all experimental procedures in detail. They

also will perform a pertinent medical history and physical examination. Normal controls and subjects with essential hypertension will be recruited via advertisement in the campus and letter of invitation (only for DHS subjects who had given consent for contact for participation in future studies). PA subjects will be recruited from the hypertension clinic at Aston ambulatory center by the P.I. and study investigators.

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9. Potential Risks:

The potential risks are related to: a) microneurography, b) intravenous infusion of nitroprusside (NTP), c) intravenous infusion of phenylephrine (PE), d) administration of eplerenone, e) venipuncture, and f) administration of amlodipine, and g) Potential risk of handgrip exercise.

a) The potential risks of microneurography: 10% of subjects may experience transient arm or legtiredness, or increase sensitivity to touch (hyperesthesia), motor weakness lasting less than 1 week. Since 1979, microneurography has been performed on more than several thousand subjects without permanent complications related to procedure.

b) The potential risks of administration of nitroprusside is flushing, hypotension and tachycardia. *For safety reasons, NTP will not be given if resting BP is ≤90/60 mmHg.*

c) The potential risk of administration of phenylephrine is excessive increase in BP. *PE will not be given if resting BP is ≥ 160/100 mmHg.*

d) Potential risk of Eplerenone is hypotension, hyperkalemia, and renal dysfunction.

e) Potential risks of venipuncture are infection, bleeding, and vasovagal reaction.

f) Potential risk of amlodipine is hypotension, gingival hyperplasia, and lower extremity edema.

g) Potential risk of handgrip exercise is hand discomfort and excessive increase in BP. However, *study will be terminated if BP during exercise at any time point is 220/105 mmHg or above for safety reasons according to the current guidelines* [10, 11].

h) Potential Risks for exercise testing: There is a finite probability of an adverse cardiovascular event (myocardial infarction or sustained ventricular tachycardia: 1.2/10,000) and death (<~1/10,000) in individuals undergoing exercise testing. Exercise testing will be terminated based on standard indications, including ST-segment elevation, decreased systolic blood pressure > 10 mmHg with exercise, sustained ventricular tachycardia. Resuscitative equipment and study personnel certified in Advanced Cardiovascular Life Support (ACLS) will be nearby in the rare case of a medical emergency. If there is cardiovascular or respiratory compromise, the “Code Blue” system will be triggered in the UT Southwestern campus while ACLS is administered by trained personnel within the room. A defibrillator is located in the exercise lab and can be immediately available should the situation arise. An attending cardiologist will be immediately available by phone call as needed.

10. Subject Safety and Data Monitoring:

a) Microneurography will be performed under the supervision of Dr. Vongpatanasin who has extensive experience. A stimulus isolation unit (special electronic circuit) will be used to prevent electrocution from the recording apparatus. Subjects will be given a microneurographic questionnaire to report any adverse reactions occurring up to one week after the study.

b) All subjects will have a routine history and physical examination performed by the physician-investigators. In addition, hospital medical records will be reviewed prior to the initiation of these studies. Venipuncture will be performed by research nurse at the CTRC, using sterile technique.

c) ACLS-certified personnel, including a board certified cardiologist, will be present during all experiments. Resuscitative equipment and drugs will be available in the laboratory. Blood pressure and the subject's heart rate will be monitored continuously during all experiments. *During baroreflex testing, NTP will not be given if resting BP is ≤90/60 mmHg and PE will not be given if BP is ≥ 160/100 mmHg.*

During handgrip exercise intervention, study will be terminated if BP at any time point is 220/105 mmHg or above for safety reasons according to the current guidelines [10, 11].

d*) During treatment with eplerenone in aim 1e, BP and serum K will be monitored every 2 weeks.* Each subject will be started with 50 mg eplerenone daily or 2.5 mg amlodipine daily. If BP remains above 140/90 mmHg at week 2, the dose of eplerenone will be increased to 100 mg daily, whereas amlodipine will be increased to 5 mg daily. If BP remained elevated at week 6, eplerenone will be increased to 200 mg daily and amlodipine to 10 mg daily *If subjects develop hypotension (BP < 90/60 mmHg persistently), the eplerenone and amlodipine will be reduced by 50%. BP will be rechecked within 2 weeks in these subjects.*

This dose range and pattern of titration of eplerenone and amlodipine is chosen because it was shown to produce equivalent reduction in BP in a large clinical trial [27] *and there were no serious adverse events related to either drug with this dose range* [27, 28]*. The incidence of hyperkalemia (K > 5.5 mmol/L) was between 1.1-3.0% in the eplerenone group* [27, 28] *and 1.5% in the amlodipine group* [27]

If subjects develop serum K between 5.5-6.0 mmol/L with eplerenone, the dose will be reduced by half and serum K will be rechecked in 2 weeks.

Those who develop hypokalemia during the follow up visit (K 3.0-3.5 mmol/L) will be started on K supplement 40 mmol daily and serum K will be rechecked in 2 weeks.

Subjects who develop severe hypokalemia (< 3.0 mmol/L) or hyperkalemia (> 6.0 mmol/L), EKG will be obtained immediately and the study procedures will be discontinued in these subjects. Subjects will be transferred to the emergency department for further evaluation and management.

Known adverse events and the likelihood of occurrence are described in the IRB-approved informed consent document.

The classification of adverse events will follow the Common Toxicity Criteria used by the National Cancer Institute at http://ctep.cancer.gov/forms/CTCv20_4-30-992.pdf. Attribution of an adverse event to study procedures will be in accordance with IRB terminology ("definite," "probable," "possible," "unknown," or "unrelated" relationship).

Ms. Beverley Adams-Huet, our collaborating investigator, will perform the aggregate analysis of adverse event reports.

In the absence of adverse events, data analysis will occur at weekly intervals and reviewed at weekly lab meetings.

Monitoring will be performed in conjunction with each subject's experience with study procedures, and conclusions of the monitoring reported to the IRB and CTRC no less than every 12 months in accordance

with IRB and CTRC requirements. Such progress reports will include a discussion of data quality and timeliness, participant recruitment, accrual and retention; participant risk versus benefit, and other factors than can affect study outcome.

Monitoring reported to the IRB and CTRC will also include consideration of factors external to the study when interpreting the data, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the study.

Stop Points: *Any of the following criteria will constitute stop points.*

- *Refractory hyperkalemia defined as more than one episode of serum potassium level > 5.5 mEq/L. The second episode must be confirmed by repeat measurement in the core laboratory.*
- *Withholding of study medication for > 4 weeks for whatever reason.*
- *Any reason including serious adverse events that results in participant's inability to continue with study protocol and procedures.*
- *Severe hypertension defined as persistent elevation of systolic BP > 180 mmHg or diastolic BP > 110 mmHg on 3 consecutive measurements after randomization*
- *Refractory hypotension defined as persistent systolic BP < 100 mmHg*
- *Pregnancy*
- *Development of diabetes mellitus or a new medical condition necessitating administration of ACE inhibitor, angiotensin receptor blocker, or spironolactone*

Conditions for temporarily halting study medications: *These include 1) sudden increase in serum creatinine of > 50% of the baseline value obtained on the CTRC visit prior to randomization; 2) Severe uncontrolled hypertension in the outpatient setting defined as elevation of systolic BP > 180 mmHg or diastolic BP > 110 mmHg on two consecutive occasions measured at least 48 hours apart despite effort to lower BP by diet or nonpharmacologic intervention.*

Conditions for permanently halting study medication in an individual: *A stop point will be defined as any serious adverse event that in the opinion of the Principal investigator could be reasonably attributed to the study intervention. At this time the participants study medication will be permanently discontinued and a close out visit will be scheduled.*

Conditions for stopping the protocol entirely: *Conditions for stopping the entire protocol are 1) development of severe hyperkalemia (serum K > 6.0 mEq/L) or acute renal insufficiency (eGFR reduces by > 30%) in 1 subject or more after they have been randomized to study drug, (2) any evidence of myocardial ischemia or infarction (chest pain, EKG changes), (2) any new onset of sustained cardiac arrhythmia including atrial fibrillation, supraventricular tachycardia, ventricular tachycardia, or ventricular fibrillation; (3) any death. Under such circumstances, the study will not resume without IRB and CTRC approval.*

Reports to Federal, University, and hospital offices

The PI will immediately report all unexpected and any serious adverse events and the recommendations derived from data and safety monitoring (such as continuation or conclusion of the trial) to the IRB, CTRC, hospital, FDA, and NIH.

The PI will immediately notify NIH of actions, if any, taken by the IRB as a result of its continuing review.

The PI will submit copies of FDA reports about adverse events to the CTRC as well as IRB.

The PI will anonymize all reports of local adverse events submitted to the IRB and CTRC to protect a subject's privacy.

The PI will educate all key personnel about the requirements to report unexpected and serious adverse events to the IRB, CTRC, and other agencies and offices.

11. Procedures to Maintain Confidentiality

1. Information will be given only to the physician-investigators.
2. The nature of the information concerns pertinent medical and social history.
3. The purpose of the disclosure is solely for research purposes, in particular to determine whether potential subjects meet criteria for inclusion or exclusion and to identify specific subgroups of subjects.
4. The subject's right of confidentiality will be given strict priority. No mention of the subjects' identities will be made either directly or indirectly in oral or written presentation of this work. The investigators, who are physicians, will be responsible for providing medical care in the event of any adverse effects to the subjects.
5. Copies of executed consent forms and all experimental records will be in the locked cabinet in our cardiovascular physiology laboratory in H4.136. All medical and biographical information will be held strictly confidential and no disclosures of personal identity will be allowed unless specifically requested by the subject. Copies of executed consent forms, as well as the experimental log book, are kept in a locked file cabinet in this laboratory.
6. Data management: The data repository will be developed using the Microsoft SQL Server relational database management system with Microsoft Access as the user interface.

Data will be centralized and maintained upon a secure centralized network server and is backed up on a rotating schedule. This system allows access to the database throughout UTSW campus university network but database privileges are only allowed on an as-needed basis and must be approved by the Principal Investigator. Data that is electronically captured will be reviewed before being merged into the database. Audit logs of all data access will be maintained for security purposes. De-identified data will be shared with collaborating investigators according to University rules and regulations.

12. Potential Benefits of the proposed Research to the Subjects and Others

Hypertensive patients are known to have exaggerated rise in BP during exercise, which poses substantial health risks independent of resting BP [5-9]. However, the underlying mechanisms of augmented exercise-induced BP elevation in human hypertension remain unknown. Our current research proposal is designed to assess the role of sympathetic nervous system and sympathetic mediated vasoconstriction during exercise in hypertensive patients, which may lead to identification of new drug target or treatment for augmented exercise pressor response.

1. Although these experiments are not designed to benefit the subjects directly, all subjects will receive monetary reimbursement for participation in the study.

2. However, the results of these studies may lead to understanding of mechanism of augmented exercise-induced BP elevation. If we can confirm that circulating aldosterone or local aldosterone synthesis in the central nervous system is the major cause of this abnormality by sensitization of mechanoreflex, our work could lead to identification of new action of MR antagonists which can prevent excessive rise in BP during exercise in hypertensive patients. This in turn will allow more hypertensive patients to participate in regular exercise.

The anticipated large amount of new knowledge about the role of sympathetic nervous system and modulation of sympathetic vasoconstriction in pathogenesis of augmented exercise pressor response should justify the performance of this research. We anticipate that the results of our human integrative cardiovascular research will provide a new conceptual framework for implicating mechanoreceptor sensitization causing reflex sympathetic activation and exaggerated exercise BP response in human hypertension. Our study may also lead to identification of the origin of accentuated mechanoreflex and exercise intolerance in heart failure.

13. Statistical Considerations SAS (SAS Institute, Cary, NC) software will be used for all analyses and PROC MIXED for repeated measures [29]. Statistical Considerations Sample size and power estimates were computed using PASS 11 (NCSS, LLC, Kaysville, UT).

The primary endpoint is muscle SNA (bursts/min, aim 2a, b, d, and e) and skin SNA (%total activity, aim 2c). Secondary endpoints include SNA % total activity (only for muscle SNA since skin SNA units are already in % total activity), MAP, and HR. In all aims, the association between SNA responses and NE, insulin resistance variables will be analyzed with Pearson or Spearman correlation coefficients.

To control for resting levels for SNA, BP, or other endpoints which could be affected by drug treatment (aim 2e) or surgical removal of tumor (aim2d), the resting or time 0 measurement prior to each activity (see fig 12-13) will be analyzed as a control covariate (analysis of covariance approach) in repeated measures models. This approach will also increase the precision of the response estimates. We will also evaluate changes and % changes from baseline levels to facilitate interpretation of the results. Repeated measures (RM) analysis will be conducted with mixed linear models with subject as a random effect. All available data will be included in the analyses, including subjects with missing data. Model parameter estimates in the presence of missing data will be made with restricted maximum likelihood. Most analyses will include a repeated factor for comparing the activity gradations; covariance structure is selected on the basis of information criteria as well as model parsimony. For multiple comparisons, adjustments will be made as appropriate with SAS's simulation adjustment method with a family-wise error rate of 0.05 and fixed seed [29].

Due to our prior success with frequency matching enrollment [25, 30-32], study groups should be balanced with respect to gender, menopausal status, ethnicity, age, and BMI and we do not anticipate needing additional covariate adjustments. However, we will assess effects of these and other potential confounding variables from e.g., 24h urine Na, descriptive statistics, scatter plots, box plots, and by modeling the covariate.

A two-period crossover study of amlodipine vs eplerenone medication will be conducted in 15 PA and 15 EH subjects. This sample size accounts for ~20% attrition and is powered for a separate crossover analysis for each group to detect a mean difference of 6 bursts/min (std=8, ρ=.7) when comparing the primary endpoint of SNA between the two medication phases. With 12 subjects completing both medication crossover phases, this study has power of 0.88 at alpha=0.05. Between group response differences are not a primary hypothesis; hence are not evaluated for power. *Randomization:* Stratified by EH and PA, the order of amlodipine and eplerenone treatment will be determined by blocked randomization. *Statistical analysis:* For each study group, descriptive statistics and 95% CI will be used to summarize responses of the two medication phases of this crossover study. A mixed linear model with repeated factors to assess the activity level and medication effect plus their interaction will be constructed. Treatment sequence effect will be evaluated in the models. To account for reduction in BP after each drug treatment, resting BP will be analyzed as baseline covariates. Other analyses include controlling for *final medication dose* as potential confounding factor and evaluating differences between the PA and EH groups with regard medication response using models that include PA and EH groups to directly and efficiently assess the group by MR antagonist interaction component.

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