Cover Page

Contribution of Substance P to Blood Pressure Regulation in the Setting of Dipeptidyl Peptidase IV (DPP4) and Angiotensin-Converting Enzyme (ACE) Inhibition

NCT02130687

Uploaded 4/17/2021

Reuploaded 5/13/2021

Contribution of Substance P to Blood Pressure Regulation in the Setting of Dipeptidyl Peptidase IV and Angiotensin-Converting Enzyme (ACE) Inhibition

PI: Nancy J. Brown, M.D.

Table of Contents:

1.0 Background

According to the 2005-2006 National Health and Nutrition Examination Survey (NHANES), approximately 65 million people in the United States have hypertension.[1] Hypertension is the most important modifiable risk factor for kidney disease, cardiovascular disease and stroke.[2-5] Comorbidities such as dyslipidemia, obesity, and insulin resistance account for additional cardiovascular risk. These cardiovascular risk factors comprise the metabolic syndrome, which in turn increases the risk of type 2 diabetes (T2DM). Diabetes has also been associated with a dramatically increased risk of heart attack, stroke, and renal failure.[6] It is estimated that more than 24 million people in the United States have diabetes. In these individuals, the prevalence of hypertension is 1.5 to 3 times greater than in sex-aged-matched controls.[7,8]

Given the additive cardiovascular risk of diabetes and hypertension, blood pressure goals in diabetic subjects are ≤ 130/80 mm Hg, as defined by the American Diabetes Association (ADA) [9] and the Seventh Joint National Committee for the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7).[10] The evidence that supports the lower blood pressure goals in diabetic patients comes from large controlled studies, such as The United Kingdom Prospective Diabetes Study (UKPDS), which demonstrated a 45% reduction in relative risk of fatal and non-fatal stroke with tight BP control, with a BP 10/5 mm Hg lower than the group randomized to less controlled.[11] Similarly, the Hypertension Optimal Treatment study showed a 50% reduction in cardiovascular disease in the group randomized to achieve a diastolic blood pressure below 80 mm Hg.[12]

In order to achieve blood pressure goals, lifestyle modifications and pharmacologic therapy are the mainstay therapies. Lifestyle modifications have proven to be effective, however only few patients are able to achieve blood pressure control with those interventions.[13] The JNC 7 guidelines recommend starting both antihypertensive medication and lifestyle modifications in patients with diabetes when hypertension is diagnosed.[10] Among the antihypertensive drugs available, angiotensin converting enzyme (ACE) inhibitors are the first-line therapy in patients with hypertension and diabetes, according to guidelines from the ADA, the NKF, the World Health Organization, and the JNC 7. [9,10,14,15]

To reduce the risk of cardiovascular, cerebrovascular disease and renal failure in patients with both diabetes and hypertension, it is also important to achieve a good glycemic control. Among the anti-diabetic agents, dipeptidyl-peptidase-4 (DPP-4) inhibitors are novel anti-diabetic agents. These drugs inhibit the DPP-4 enzyme that degrades the incretin hormones GLP-1 and GIP, which stimulate insulin release in response to an enteric glucose load.[16] The first DPP-4 inhibitor approved by the FDA was sitagliptin. Since its approval in 2006 this drug has become one of the leading branded oral anti-diabetic agents in the United States.[17] DPP-4 inhibitors offer advantages over commonly used anti-diabetic agents, such as a glucose-dependent mechanism of action and lack of weight gain.[18,19,20] However, there is information that remains to be known regarding safety, efficacy and drug interactions of DPP-4 inhibitors.

2.0 Rationale and Specific Aims

Hypertension and diabetes are important modifiable morbidity and mortality risk factors. The prevalence of hypertension is nearly 1 billion worldwide.[21] On the other hand; approximately 246 million people have diabetes worldwide.[22] Among the latter the incidence of hypertension is 1.5 to 3 times higher than in sex and age matched control.[7] The first line therapy in patients with diabetes and hypertension are angiotensin converting enzyme (ACE) inhibitors because of its renoprotective and antithrombotic properties. Despite the wide variety of anti-diabetic drugs available, it remains difficult to achieve adequate glycemic control. DPP-4 inhibitors are a promising new anti-diabetic drug class. By inhibiting the degradation of the DPP-4 enzyme, they prevent the degradation of incretins GLP-1 and GIP, which increase the release of insulin, suppress glucagon release, and delay gastric emptying.[16]

Notably, DPP-4 enzyme intervenes in the degradation of several vasoactive peptides such as brain natriuretic peptide (BNP), Neuropeptide Y (1-36), Peptide YY (1-36), and substance P (SP).[23] The ubiquitous nature of this enzyme would translate into diverse hemodynamic effects, and could potentially result in diverse drug interactions, as it was ascertained by Jackson et al. They found that the DPP-4 inhibitor, P32/98, significantly increased blood pressure in pre-treated (captopril 30 mg/kg or hydralazine 5mg/kg) adult spontaneous hypertensive rats (SHR).[24] Interestingly this effect was abolished by effective ganglionic blockade with chlorisondamine, suggesting that activation of the sympathetic nervous system was determinant for the increased blood pressure seen in the SHR model.[24] The hypertensive effect induced by the DPP-4 inhibitor in that study was attributed to neuropeptide Y; nevertheless a hypertensive effect mediated through other vasoactive peptides, such as substance P could not be ruled out.

Substance P on the other hand, is primarily inactivated by angiotensin-converting enzyme (ACE); under ACE inhibition however, DPP-4 becomes the major degradation pathway of this peptide.[25] Therefore, the interactive inhibition of DPP-4 and ACE will theoretically result in a significantly impaired degradation of substance P. Furthermore, ACE inhibition decreases the degradation of bradykinin which in turns increases the release of substance P by sensory nerves.[26] This has been associated with an increased risk of ACE inhibitor-associated angioedema in patients treated with the DPP-4 inhibitor, vildagliptin.[27] It is important to note that although SP induces vasodilation by acting on NK1 receptor,[26] it does not appear to decrease peripheral vascular tone, or systemic blood pressure.[28]

We have previously assessed the hemodynamic effects of the concomitant inhibition of the DPP-4 enzyme and the ACE in subjects with metabolic syndrome. The main finding of the study was that sitagliptin prevented the decrease in mean arterial blood pressure (MAP) when ACE was fully inhibited by enalapril, but not during sub-maximal ACE inhibition. Furthermore, the HR significantly increased in response to 10 mg enalapril plus sitagliptin. This was also associated with an increase in plasma catecholamine levels, which suggested an increased sympathetic outflow.[29] We hypothesize that these effects on blood pressure, heart rate, and sympathetic activity could be due to an unrestrained amount of substance P, secondary to the inhibition of the two main enzymes involved in its degradation.[29]

Specific Aim: To test the hypothesis that DPP4 inhibition attenuates the anti-hypertensive effect of chronic ACE inhibition, but not AT1 receptor inhibition, in T2DM through a substance P (NK1) receptor-dependent mechanism

3.0 Animal Studies and Previous Human Studies

Substance P is a member of the tachykinin family peptides, which interact with neurokinin-1 (NK_1), NK_2, NK_3 receptors.[34] Its effects however, are mainly mediated by NK_1 receptor. This peptide is widely distributed in enteric, peripheral, and central nervous systems. SP is involved in a wide variety of mechanisms, such as pain perception, nociception, emesis, and inflammatory responses.[35,36] The evidence suggests that SP is also involved in cardiovascular regulation. Numerous animal studies have described the effect of substance P in the nucleus of the tractus solitarious (NTS) and its effect in the modulation of cardiovascular function.[37,38,39,40]

Furthermore, Comet et al analyzed the effect of intra-NTS administration of the selective NK_1 receptor antagonist, GR205171, on baroreflex bradycardia inhibition observed during the defense reaction triggered by electrical stimulation of the dorsal periaqueductal grey matter in anesthetized rats. The bilateral microinjection of SP in NTS produced an immediate and brief (6-7 min) increase in MBP (+25±2 mm Hg from a baseline of 84±1 mm Hg, $P<0.05$) and a concomitant decrease in HR (-45±7 bpm from a baseline of 334±5 bpm, $P<0.05$). Moreover, bilateral microinjections of SP into the NTS produced a dose-dependent inhibition of phenylephrine evoked-cardiac response and aortic cardiac response which was reversed by the selective NK1 receptors antagonist, GR205171.[38]

Scheneider et al demonstrated that the intraventricular injection of substance P in Wistar rats induced a hypertensive effect.[41] SP increased MAP and HR +31.4±2.5 mm Hg and +190±13.7 bpm from baseline, respectively. An increase in plasma cathecolamines was also apparent. Interestingly, these effects were significantly attenuated in a dose-dependent manner by the administration the GABA agonist, muscinol.[41] Similarly, the microinjection of substance P in the nucleus of the amigdala centralis also elicits pressor responses, whereas pre-injection with [D-Pro^2, D-Phe^7, D-Trp^9]-SP, a substance P antagonist, in the same area attenuated the pressor response to glutamate.[42]

The mechanism underlying the pressor actions and heart rate responses are attributed to sympato-adrenal stimulations, as demonstrated by pharmacologic testing and sympathetic nerve recording.[30,31,32] Unger et al reported increased BP, HR, and sympathetic nerve activity (splenic, renal, and adrenal nerves) in conscious rats. They found that the acute administration of 1 µg of substance P increased the sympathetic nerve activity by + 85%, + 147%, and + 63%, respectively ($P < 0.001$).[32] In a similar fashion, Dzurik et al demonstrated that substance P increases

sympathetic outflow in humans, as evidenced by a decrease muscle sympathetic nerve activity (MSNA) with the administration of the NK-1 receptor antagonist, aprepitant.[33]

We have previously reported that the DPP-4 inhibitor, sitagliptin, attenuates the hypotensive effect of enalapril (10 mg) in patients with metabolic syndrome. Mean arterial blood pressure changes were -7.9±2.4 mm Hg with placebo plus enalapril, whereas MAP was -0.9±0.9±2.3 mm Hg with sitagliptin plus enalapril. Interestingly, this effect was only evidenced with 10 mg of enalapril, but not with 5 mg. Moreover, the HR significantly increased in response to 10 mg enalapril plus sitagliptin. This was also associated with increased in plasma catecholamine levels, which suggests an increased sympathetic outflow.[29] We proposed that the effects on blood pressure, heart rate, and sympathetic activity could be due to an unrestrained amount of substance P, secondary to the inhibition of the two main enzymes involved in its degradation.[29]

4.0 Inclusion/Exclusion Criteria

Inclusion criteria

- Age 18 to 80 years old.
- For female subjects the following conditions must be met:
  - Postmenopausal status for at least 1 year, or
  - Status post-surgical sterilization, or
  - If of childbearing potential, utilization of barrier methods of birth control and willingness to undergo urine β-HCG testing prior to drug treatment and on every study day (oral contraceptive medication will not be accepted as a sole birth control method given that aprepitant can decrease the effects of this birth control method)
- T2DM, as defined by one or more of the following,
  - Hgb A1C ≥6.5%, or
  - Fasting plasma glucose ≥126mg/dL, or
  - 2-hour plasma glucose ≥200 mg/dL following 75gr oral glucose load

- Hypertension, as defined by:
  - Seated SBP ≥130 mm Hg on three occasions (documented in medical record), or
  - Seated DBP ≥80 mm Hg on three occasions (documented in medical record), or
  - Treatment with antihypertensive medications for a minimum of 6 months

Exclusion criteria

- Type 1 diabetes.
- Poorly controlled T2DM, defined as Hgb A1C>8.7%.
- Use of anti-diabetic medications other than metformin for at least 12 months prior to initiation of the study.
- Secondary hypertension.
- Subjects who have participated in a weight-reduction program during the last 6 months and whose weight has increased or decreased more than 5 kg over the preceding 6 months.

- Pregnancy. Breast-feeding.
- Treatment with any of the following drugs: cisapride, pimozide, terfenadine, astemizol
- Clinically significant gastrointestinal impairment that could interfere with drug absorption
- Cardiovascular disease such as myocardial infarction within 6 months prior to enrollment, presence of angina pectoris, significant arrhythmia, congestive heart failure (LV hypertrophy and diastolic dysfunction acceptable), deep vein thrombosis, pulmonary embolism, second- or third-degree AV block, mitral valve stenosis, or hypertrophic cardiomyopathy
- Impaired hepatic function (aspartate amino transaminase [AST] and/or alanine amino transaminase [ALT] >3 x upper limit of normal range)
- Impaired renal function (eGFR< 50mL/min/1.73m^2 as determined by the MDRD equation).
- History or presence of immunological or hematological disorders.
- History of pancreatitis or known pancreatic lesions.
- History of angioedema while taking an ACE inhibitor.
- Hematocrit <35%.
- Treatment with anticoagulants.
- Diagnosis of asthma requiring use of an inhaled β-2 agonist more than 1 time per week.
- Any underlying or acute disease requiring regular medication which could possibly pose a threat to the subject or make implementation of the protocol or interpretation of the study results difficult
- Treatment with systemic glucocorticoids within the last 6 months.
- Treatment with lithium salts
- Treatment with any investigational drug in the 1 month preceding the study
- Mental conditions rendering the subject unable to understand the nature, scope, or possible consequences of the study
- Inability to comply with the protocol, e.g., uncooperative attitude, inability to return for follow-up visits, and unlikelihood of completing the study

5.0 Methods and Protocol

Recruitment of subjects

Subjects will be recruited from the Vanderbilt outpatient clinics identified through Subject Locator (Starbrite/StarPanel), MyResearch@Vanderbilt, the research derivative, as well as from a pool of study volunteers who have identified themselves to the Vanderbilt University Clinical Research Center (CRC) as interested in studies involving T2DM and/or hypertension. Written advertisements approved by the Vanderbilt Institutional Review Board (IRB) will be placed on Vanderbilt and community bulletin boards. Contact information for the study will be placed on the advertisements. Subjects who call for information will be given a brief description of the study protocol and, if interested, will be invited to the Vanderbilt CRC for more information. During this meeting the research nurse and/or investigator will describe the study protocol in detail and answer all questions. Interested subjects will be invited to read and sign an IRB-approved consent form and will be given a copy of that consent form to take home.

Enrollment and Randomization

Figure 1 illustrates the randomized, double-blind, placebo-controlled study protocol. Subjects who meet inclusion and exclusion criteria will be withdrawn from all anti-hypertensive medications that affect measurements of mechanisms of interest in the study for three weeks (i.e., beta blockers, clonidine, etc). Hydrochlorothiazide (HCtZ) may be continued throughout the study at a maximum dose of 25 mg. Because we have found that spironolactone requires longer washout, this medicine will be discontinued 4 weeks prior to initiation of study drug. If appropriate, medications will be tapered. During this period, blood pressure will be measured every 1-3 days. If at any time the seated SBP is >170 mmHg, or the seated DBP is >110 mmHg, or if a subject develops symptoms of high blood pressure regardless of the blood pressure, that subject will be discontinued from the study, and his or her anti-hypertensive medications will be restarted.

Following washout, we will randomize subjects in a 1:1:1 ratio to receive pre-treatment with ramipril 5mg/d for 3 days and then 10mg/d, or valsartan 160mg/d for three days and then 320mg/d, or amlodipine 5mg/d for 3 days then 10mg/d for a total of 15 weeks. We will give the first dose of study medication at the CRC. We will continue to monitor blood pressure every 1-3 days during the first week of randomization and then every week throughout the study.

Following 4 weeks of pre-treatment with antihypertensive medication, each subject will additionally receive three different 1-week concurrent interventions, in a cross-over fashion, separated by at least a 4-week washout. The interventions will be: placebo + placebo, sitagliptin 100mg/d + placebo, and sitagliptin 100 mg + aprepitant (125 mg on the first day followed by 80mg/d). A minimum of five half-lives between each intervention is necessary to prevent a carry-over effect. Sitagliptin and aprepitant have half-lives of 12 and 9-14 hours, respectively. Therefore, subjects will be required to undergo at least a 4-week washout between each intervention (**Figure 1)**. The study days will take place on the last day of each intervention.

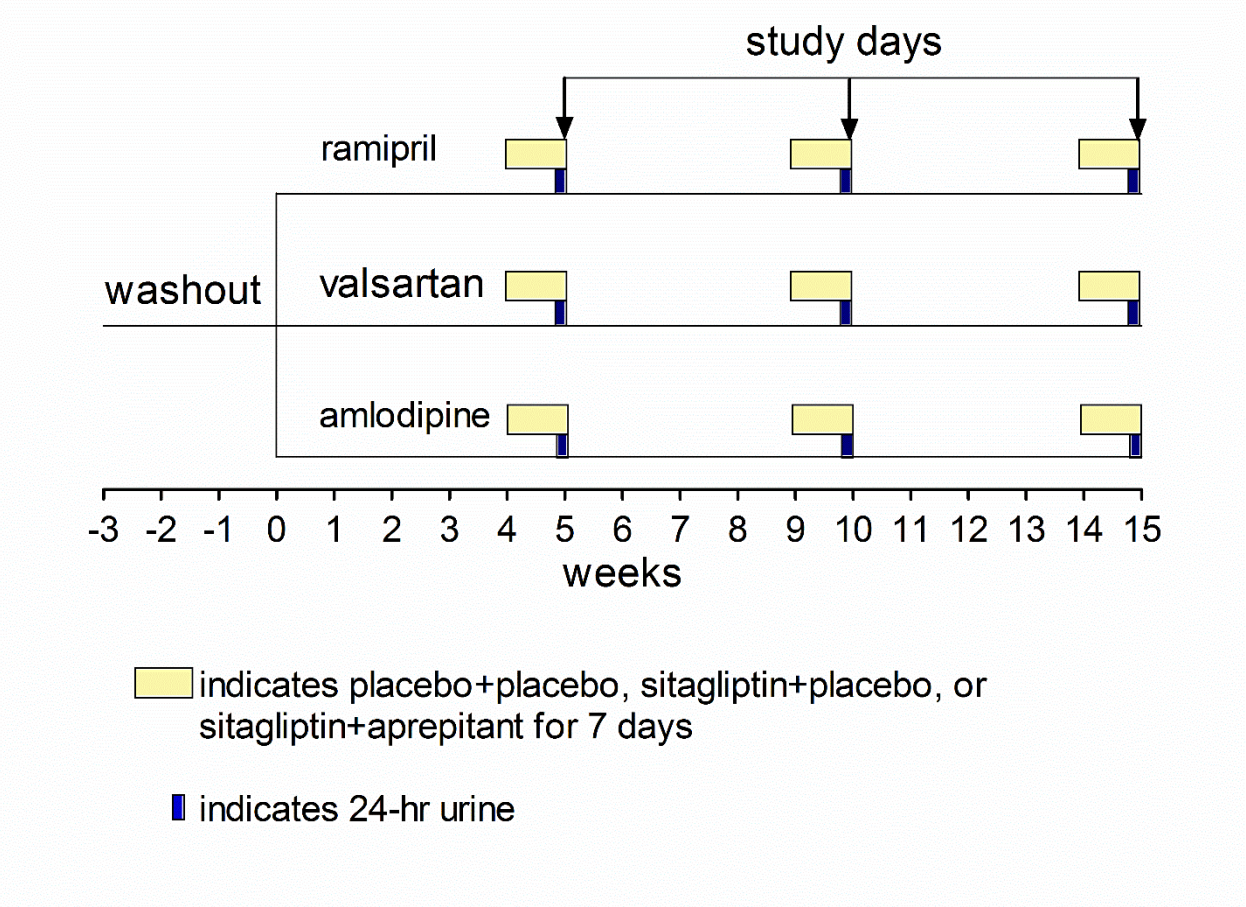

Figure 1. Study Protocol.

The Vanderbilt Investigational Drug Service will be responsible for the storage, preparation, and labeling of all investigational agents and for maintaining accurate drug storage and dispensing logs. At the time of randomization the research nurse will fax a copy of the consent form and a prescription containing check boxes for inclusion and exclusion criteria. The pharmacist will assign the subject a randomization number and provide the investigator a 15-week supply of pre-treatment drug along with a 1-week supply for the first of the three interventions. On study days 1 and 2 the pharmacy will provide the investigator with a 1-week supply with the remaining two interventions. The tablets will have an identical appearing form. An extra label containing the randomization number will be attached to each bottle of study drug. The investigator will affix this extra label to the subject's records. The Investigational Drug Service will retain a secure set of sealed envelopes containing the treatment assignment. Those envelopes will be opened in the event of a clinical scenario that compromises the safety of the subject, in which unblinding will alter the clinical outcome, as determined by the PI and the data safety monitoring committee.

Blinding

This study will be double–blinded. Neither the investigator nor the patient will be aware of the treatment assignment or the order of randomization.

6.0 Endpoints

The primary analyses will focus on:

MAP, SBP, DBP, heart rate, and NE concentrations during ramipril versus ramipril+sitagliptin
MAP, SBP, DBP, heart rate, and NE concentrations during ramipril+sitagliptin versus ramipril+sitagliptin+apreptitant.

We will make similar comparisons within the valsartan- and placebo-treated groups. In addition, we will compare MAP, SBP, DBP, heart rate, and endocrine parameters among the ramipril-treated, valsartan-treated, and amlodipine-treated groups during comparable concurrent treatment. Secondary endpoints of the study will include:

Hemodynamic and renal measurements: urinary sodium, potassium, and catecholamine excretion
Vasoactive biomarkers: substance P, substance P metabolites, neuropeptide Y, DPP-4 activity, ACE activity, plasma renin activity (PRA), angiotensin II, aldosterone, plasma catecholamine levels
Glucose homeostasis biomarkers: plasma glucose, plasma insulin, and glycerol

7.0 Study Procedures

Screening Visit 1: After informed consent is obtained, subjects will undergo a medical history and physical examination. During this and all visits to the CRC we will measure blood pressure three times, every two minutes as described under **Standard Techniques**. We will measure weight, height, and hips and waist (horizontal umbilicus) circumference to 0.5 cm precision in triplicate. We will use a spring-loaded tape measure (Gulick II by Country Technology, Gay Mills,WI) for these measurements to ensure the tightness of the tape is consistent. We will obtain screening laboratory tests including: electrolytes, serum creatinine, fasting glucose level, complete blood count, liver enzymes, lipid profile, hemoglobin A1C, and urinalysis. Data will be transferred using the REDCap Dynamic Data Pull (DDP) to enable transfer of relevant study related data directly from the Vanderbilt Research Derivative.

Screening Visit 2: subjects whose screening labs are adequate will return to the CRC to undergo an oral glucose tolerance test (OGTT).

Medication withdrawal: Hypertensive subjects will have appropriate anti-hypertensive medications (those of which have the potential to affect measurements of interest during the study) discontinued three weeks prior to the initiation of the study, **Figure 1**. Spironolactone will be discontinued four weeks prior to initiation of study drug. Hydrochlorothiazide may be continued throughout the study at a maximum dose of 25mg daily. If appropriate, medications will be tapered. During this period, subjects will be asked to monitor their blood pressure every 1-3 days for the

first week after randomization. Subjects will then be asked to monitor their blood pressure every week. We will provide the subjects with a manual sphygmomanometer and a BP diary for them to record their BP. The research nurse will contact them every 3 to 5 days to ensure compliance. Subjects will also be instructed to contact the research nurse and/or investigator if at any time their seated systolic pressure is >170 mmHg or their seated diastolic pressure is >110 mm Hg. Those subjects will be immediately withdrawn from the study and will resume anti-hypertensive medication. Excluded subjects will be replaced.

Subjects on oral diabetes medications other than metformin will have these medications discontinued for three weeks prior to initiation of the study. Subjects will be asked to monitor their blood glucose using a home glucometer every 1-3 days for the first week and at least weekly thereafter. Subjects will be instructed to contact a member of the study team if their glucose level is ≤70mg/dL or ≥300mg/dL. Should this occur and the subject have sustained hyperglycemia >300mg/dL or significant hypoglycemia at any time, the subject will be discontinued from the study and his/her regular medications will be restarted.

Medication Visit: Subjects will be asked to come to the CRC at least 30 days before study day 1 to pick up the study medications. We will request a urine sample for urinary β-HCG in female subjects of childbearing potential. Patients will be instructed on how to take the study drugs. The first of the three concurrent interventions will be provided to subjects 6 days prior to study day 1. We will provide the subject with a urine jug to provide a 24-hour urine collection to measure urinary sodium, potassium, and catecholamine excretion. A 24-hour urine sample will be collected during each study day. We will provide subjects with a medication diary to write down any additional medication started after screening for the duration of the study.

Study days During each study day subjects will be asked to report to the CRC in the morning in a fasting state to undergo serial blood pressure and heart rate measurements. Subjects will bring the 24-hour urine collection and medication diary. Additionally, women of childbearing potential will be asked to provide a urine sample for β-HCG. Subjects will be asked not to exercise for at least 3 days prior to each study day and avoid alcohol and caffeine intake starting 1 week prior to each study day.

An indwelling IV catheter will be placed in the dominant arm. A blood pressure cuff will be placed on the non-dominant arm. After instrumentation subjects will be asked to rest quietly in supine position for 30 minutes. Subjects will then undergo power spectral analysis as described under **Standard Techniques**. With apparatus in place the subject will received the pre-treatment and intervention drug by mouth with a small amount of water (t=0). Blood samples for vasoactive biomarkers will be drawn at baseline and every hour thereafter for 4 hours. We will draw blood for measurement of glucose, insulin, DPP4 and ACE activity, plasma renin activity (PRA), Ang II, and aldosterone at baseline and 3 hours. We will measure substance P, NPY, and catecholamines [NE, epinephrine, and the intraneuronal metabolite dihydroxyphenyl glycol (DHPG)] prior to medication administration, every hour thereafter and after 30 min of standing.

Blood pressure and heart rate measurements will be recorded every 5 minutes for 4 hours as described under **Standard Techniques**. After completion of the 4-hour period the subject will be asked to stand up and remain in an upright position for 30 minutes (orthostatic challenge). BP and

HR will be recorded every 5 minutes throughout the 30-minute orthostatic challenge. At the end of the orthostatic challenge, we will take blood samples to measure vasoactive biomarkers (while in upright position, during maximal sympathetic activation).

Following completion of study days 1 and 2 the research nurse or physician will provide each subject with a 1-week supply of the next intervention drug, a medication diary, and a urine jug. The research nurse or physician will provide verbal and written instructions about the appropriate way to take the medication, indicating that before starting the next intervention the subject should undergo at least a 4-week washout period while continuing taking the pre-treatment drug (amlodipine, ramipril, or valsartan). Telephone or email follow-up will occur 1 and 7 days after each study day.

DNA samples: A blood sample for DNA analysis will be drawn once during the study and will be stored for analysis of genetic markers related to subject's susceptibility to the development of hypertension, cardiovascular disease, metabolic alterations, and/or response to treatment. This sample is not required for participation in the study. Genetic information will be linked with data collected during the conduct of this trial. As the relationships between genes and diseases become less equivocal, it might be possible to predict an individual's susceptibility to a disease, its prognosis, and the response to treatment. Blood specimens will be coded. Codes will not include any identification information; such as name, date of birth, or medical record number. Only study key personnel will have access to those codes. The identity of each code will be kept in a file in an encrypted computer.

8.0 Risks of Investigational Agents/Devices (side effects)

Risks/Inconvenience

- Insertion of venous catheters may cause bleeding, bruising, or infection.
- Frequent blood draws can cause anemia.
- Collecting urine in a jug for 24 hours can be inconvenient for subjects.
- Spending study days at the CRC can be inconvenient for subjects.
- There is a risk of high blood pressure during the 3-week washout (of anti-hypertensive treatment) phase of the study.
- It has been reported that vildagliptin, a DPP-4 inhibitor, increases the risk of angioedema in patients who are taking ACE inhibitors. In clinical trials the mean exposure to vildagliptin prior to the development of angioedema was 5.5 months. During the study the subjects will receive sitagliptin during two 1-week interventions, separated by at least 4 weeks. The half-life of sitagliptin is 12 hours, therefore it is unlikely that subjects develop angioedema.
- Sitagliptin can cause hypoglycemia, peripheral edema, or nausea. In addition, sitagliptin can rarely increase the risk of pancreatitis; however, this is not likely to occur given the short duration of the study and the exclusion of subjects with past medical history of pancreatitis.
- The FDA is warning that dipeptidyl peptidase-4 (DPP-4) inhibitors such as sitagliptin, saxagliptin, linagliptin, and alogliptin may cause joint pain that can be severe and

disabling. After the patients discontinued the DPP-4 inhibitor medicine, their symptoms were relieved, usually in less than a month. Some patients developed severe joint pain again when they restarted the same medicine or another DPP-4 inhibitor.

- Aprepitant can produce weakness, fatigue, nausea, constipation, hypotension, bradycardia, dizziness, dehydration, diarrhea, dyspepsia, or impaired liver function.
- Ramipril or valsartan theoretically increases the risk of hypotension; although, generally those drugs do not lower blood pressure significantly in individuals with normal blood pressure. In rare cases, ramipril or valsartan may cause hyperkalemia. However, this is not expected in subjects with normal renal function.

Protection against risk

- The research nurse or physician will be present at all times during the study days.
- Subjects with a hematocrit less than 35% will be excluded from the study.
- In hypertensive subjects who are withdrawn from anti-hypertensive medications, blood pressure will be measured every 1 to 3 days for the first week and weekly thereafter. If at any time the seated systolic pressure is >170 mmHg or the seated diastolic pressure is >110 mmHg or if a subject develops symptoms of high blood pressure, the subject will be withdrawn from the study and his/her anti-hypertensive medications will be resumed.
- Subjects will monitor their blood glucose once every 1-3 days initially, then at least weekly thereafter and notify the study personnel if their glucose levels are ≤70 or ≥300 mg/dL and symptomatic or sustained, he/she will then be discontinued from the study and restarted on his/her regular medications.
- Potassium levels will be measured during each study day to insure the subject is not hyperkalemic.

Study Withdrawal/Discontinuation

If at any time during the study the seated systolic pressure is >170 mmHg or the seated diastolic pressure is >110 mmHg or the subject develops symptoms of high blood pressure, he/she will be withdrawn from the study and his or her anti-hypertensive medications will be restarted. If in the opinion of the investigator a subject is non-compliant, the subject will be withdrawn from the study. Subjects who are withdrawn will be followed until blood pressure has returned to normal and/or symptoms has resolved.

Similarly, if at any time the study subject has sustained hyperglycemia >300mg/dL or significant hypoglycemia <70mg/dL, the subject will be withdrawn from the study. The subject will be followed until he/ she resumes his/ her prior diabetes medication(s).

9.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

The Principal Investigator will closely oversee the protocol in conjunction with the assigned research nurse. Any adverse events or toxicities that occur while subject are enrolled in the study will be reported to the IRB as per IRB guidelines. Any untoward medical event will be classified as an adverse event, regardless of its causal relationship with the study. An adverse event it is considered to be serious if it results in: death, life-threatening condition, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability or incapacity, or results in a congenital anomaly or birth defect. Serious adverse events will be reported to the Data and Safety Monitoring Committee (DSMC) and the IRB as soon as practicable following the event. Non-serious adverse events will be reported at the time of continuing review.

The PI will be responsible for submitting summary reports to the IRB annually. The report will include: the number of adverse events and an explanation of how each event was handled, the number of complaints and how each complaint was handled, the number of subject withdrawals with an explanation of why the subject withdrew or was withdrawn, and the number of instances of noncompliance with the protocol with explanation. Before submission, the report will be reviewed internally by the PI.

The DSMC will provide objective review of treatment results as they relate to human safety. The committee will be comprised of Marie Griffin, MD, Professor of Health Policy; Alvin C. Powers, MD, Director of the Vanderbilt Diabetes Center, Professor of Medicine and Joe C. Davis Professor of Biomedical Science; and Dr. Naji N. Abumrad, John L. Sawyers Professor of Surgery and Chairman, Vanderbilt Department of Surgery. Dr. Abumrad will chair the committee.

The DSMC will meet at least 3 times, once to review the protocol and twice to receive reports of the progress of the study, at the end of the first year and at the end of year two. These reports will provide information regarding safety. The committee will assess safety data including hypotension, common adverse events, hospitalizations, and other serious adverse events. During regularly scheduled meetings, the DSMC will also be provided with a list of non-serious adverse events. Interim data will be provided to the committee by Drs. Brown and Yu. The DSMC may choose to become unblinded; however, it is expected that such unblinding would not occur without reasonable concern related to either patient safety or data validity.

10.0 Statistical Considerations

Sample Size and Power Calculation

Sample size was calculated based on the ability to detect a difference in MAP measured in the same subjects during treatment with ramipril+placebo versus ramipril+sitagliptin and between ramipril+sitagliptin versus ramipril+sitagliptin+aprepitant. In our prior study of the effect of sitagliptin on the hypotensive response to 10 mg enalapril, sitagliptin increased MAP following enalapril ~7 mmHg to 95±11.6 mmHg.[25] Assuming a standard deviation of 11.6 and a correlation of 0.5 between two repeated measurements in the same subject, a sample size of 45 would provide 80% power to detect a 5-mmHg difference in MAP between ramipril and ramipril+sitagliptin or

between ramipril+sitagliptin and ramipril+sitagliptin+aprepitant. We have chosen to enroll 50 subjects per arm to allow us to detect a difference between the effect of sitagliptin in the ramipril group versus in the valsartan group. With 50 subjects in each group, we will have 72% and 84% power to detect a 6- and 7-mmHg difference, respectively.

Data Analysis Plan

We will use standard graphing and screening techniques to detect outliers and to ensure data accuracy. We will assess continuous outcomes for normality. If normality is violated, we will apply data transformation or consider non-parametric analysis methods. We will provide summary statistics for both numerical and categorical variables by study arm. We will assess comparability among randomization groups. We will conduct these basic data analysis in all three Aims.

We are using an orthogonal Latin square design to conduct a 3x3 crossover study within each of three parallel treatment arms. Although we have designed the study to avoid a carryover effect, we will use general linear models (GLM) to evaluate for the possibility of carryover or period effect as detailed in Chapter 5 (pages 212-213) of Jones and Kenward.[44] If there is evidence for a carryover effect, we will model and estimate first-order carryover effect explicitly in the following described models. A 3X3 crossover design enables us to estimate such carryover effect and to separate it from the treatment effects of interest. We will utilize mixed-effects models to analyze data with a random subject effect and with treatment factor 1 (amlodipine, ramipril, valsartan) and treatment factor 2 (placebo, sitagliptin, sitagliptin+aprepitant) as fixed effects. We will use an autoregressive model of order 1 [AR(1)] or other plausible covariance structures for the error covariance. We will utilize mixed-effects models to evaluate treatment factor 2 (placebo, sitagliptin, sitagliptin+aprepitant) within each treatment group. We will conduct additional sub-analyses such as GLM to compare the effect of treatment factor 1 (ramipril, valsartan, and amlodipine) using data collected during the placebo or sitagliptin period of treatment factor 2. Both GLM and mixed-effects models provide the flexibility of controlling for and of evaluating covariates, including gender, race, blood pressure, and prior ACE inhibitor or ARB use.

In addition to performing regression analyses, we will estimate direct treatment differences as within-subject mean difference and 95% confidence intervals. We will then compare within-subject differences (such as ΔMAPsitagliptin-placebo) between amlodipine, ramipril, and valsartan treatment groups using a two-sample t-test. If normality of the data is in question, we will utilize corresponding nonparametric tests. We will test all hypothesis at the level of α=0.05. We will use SPSS for Windows (Version 22.0, SPSS, Chicago) and the open source statistical package R (version 2.12, R Development Core Team, 2006) for analyses.

Because subjects who drop out will be replaced and based on prior experience, missing data will be unusual. Nevertheless, if data are missing for a particular time point, mixed-effects models are robust in that subjects with missing data at some time points can be included to estimate effects of interest. In addition, we will conservatively impute missing data to perform corroborative analyses with and without missing data.

Interim analysis

One interim analysis is planned when 18 subjects have been completed in each arm. O'Brien and Fleming's boundaries will be used for stopping. Specifically, the null hypothesis will be rejected at the level of 02001 in the interim analysis and at the level of 0.0495 in the final analysis. The overall type-I error will be 0.05.

Data Management and Quality Control

We will use the Vanderbilt REDCap system to design an electronic data collection form. This form will be tested before its use. The form allows for direct data entry by study key personnel and will be designed to minimize the amount of erroneous values. Clinical data, including clinical laboratory, will be entered by the research nurse. Research laboratory data will be entered by the research assistant. A unique identification case number will be used to protect the confidentiality of the study participants. The electronic data collection system will allow us to monitor the quality performance by tracking the missing data. Before analysis raw data will be reviewed to assess its accuracy and completeness.

11.0 Privacy/Confidentiality Issues

Clinical data, including clinical laboratory data, will be accessible only to key study personnel. A unique identification case number will be used to protect the confidentiality of the study participants. The case numbers and participants names will be included in the protected source Redcap database, accessible only to members of the research team. For statistical analysis purposes the information will be de-identified, and only case numbers will be included.

12.0 Follow-up and Record Retention

All records will be retained for 7 years following publication of the data. After that time, records may be archived for an additional 5 years and then shredded.

13.0 Standard Techniques

Blood Pressure Measurements: During screening, washout, and active treatment outpatient blood pressure will be measured with an aneroid sphygmomanometer (Welch Allyn, Skaneateles Falls, NY), using the appearance and complete disappearance of the Korotokoff sounds (K1 and K5) as SBPs and DBPs. The mean of three supine measurements will be used. During study days, blood pressure will be measured every 5 min using an automated oscillometric recording device (Dinamap, Critikon, Carlsbad, CA).

Power Spectral Analysis: Blood pressure fluctuates with a 10-second periodicity, and these fluctuations are commonly termed “Mayer” waves. The low frequency variability (LF_{SBP}) is thought to reflect sympathetic regulation of vasomotor tone. LF_{SBP} is increased by maneuvers that induce sympathetic activation, such as upright posture, lower body negative pressure, or infusion of depressor substances[47] and correlates with direct measurements of sympathetic traffic using

microneurography. A derivative-threshold algorithm provided the continuous series of RR intervals from ECG signal, continuous non-invasive BP from finger arterial pressure (Finapress 2300, Ohmeda, Madison, WI), and respiratory rate will be recorded. All the data recorded will be used for determination of spectral analysis. Beat-to-beat data will be digitized using the WINDAQ data acquisition system (DI220, DATAQ, Akron, OH, 14 Bit, 1000Hz) and processed off-line using a custom-written software in PV-Wave language (PV-wave, Visual Numerics Inc., Houston, TX). Detected beat-to-beat values of R-R intervals and blood pressure values will be interpolated and low pass filtered (cutoff 2 Hz). Data segments of interest are used for spectral analysis. Linear trends will be removed, and power spectral density will be estimated with the FFT-based Welch algorithm. The power in the frequency range of low frequencies (LF: 0.04 to <0.15 Hz), and high frequencies (HF: 0.15 to < 0.40 Hz) will be calculated according to the Task Force recommendations.[48]

Assays:

All blood samples will be centrifuged for 20 minutes immediately following blood drawing. Plasma will be storage at -80°C until analysis.

Substance P and NPY: Plasma samples will be collected in a protease cocktail inhibitor containing an aminopeptidase P inhibitor (apstatin), DPP4 inhibitor (vildagliptin), serine protease inhibitor (phenylmethylsulfonyl fluoride), ETA, and phosphoramidon to avoid ex vivo degradation of the peptides. NPY will be concentrated from plasma by immunoextraction using an anti-NPY monoclonal antibody (NPY02) coupled to magnetic beads, followed by C18 micro-extraction (Ziptip, Millipore). Recovery (~70%) will be calculated using 13C, 15N-labeled internal standards for NPY and NPY (3-36). NPY and NPY (3-36) will be quantified using liquid chromatography-mass spectrometry (LC-MS/MS) with ultra-high-pressure liquid chromatography (UPLC, Waters Xevo TQS) using a modification of Dr. Grouzmann's previously published method that allows detection of physiologic concentrations NPY and its metabolites in plasma. We have previously used HPLC to separate substance P from substance P (3-11) prior to assay, but this method is labor intensive. For this reason we have recently developed a method using immunoaffinity extraction using magnetic beads [kit (LSKMAGN01) from EMD Millipore] followed by UPLC-MS/MS. The absolute limit of detection of substance P is 5 pg injected. The sensitivity in plasma is currently less than this (500 pg/mL) due to ion suppression. We are working with our colleagues in the Mass Spectrometry Core and expect to resolve this, but an alternative approach remains to use HPLC separation followed by enzyme-linked immunosorbent assay (ELISA, Abcam, Cambridge, MA).

RAS parameters: We will measure PRA (DiaSorin, Stillwater, MN),90 Ang II (drawn in a cocktail of protease inhibitors; Nichols Institute Diagnostic, San Clemente, CA), and aldosterone [MP Biomedicals, Irvine, CA utilizing 125I, with a primary antibody to aldosterone (NIDDK National Hormone and Peptide Program, Torrance, CA) and a secondary gamma globulin antibody (Linco Research, St. Charles, MO)] by radioimmunoassay. We will measure ACE (Olympus AU400/AU600, Alpco Diagnostics, Salem, NH) and DPP4 activity by kinetic analysis.

Plasma catecholamines: Catecholamines will be quantified after batch alumina extraction by high-performance liquid chromatography (HPLC) using electrochemical detection. The concentrations of [3H]NE in plasma and the infusate will be measured in fractions of the column effluent corresponding to the retention time of NE. Fractions will be collected in scintillation vials and the 3H activity assayed by liquid scintillation counting (LS 6000IC, Beckman Instruments Inc).

Glucose, C-peptide, and Insulin: Plasma glucose will be measured by the glucose oxidase method with a YSI glucose analyzer (YSI Life Sciences, Yellow Springs, OH). Plasma insulin concentrations will be determined by RIA. The insulin assay cross-reacts with 38% intact human pro-insulin and with C-peptide, ≤0.01%. Samples for C-peptide and glucagon will be drawn into heparinized tubes containing 250 KIU Trasylol (Aprotinin) per mL of whole blood. This will result in a final concentration of approximately 500 KIU Trasylol per mL of serum or plasma. C-peptide will be measured using RIA (Millipore).

Appendix A: Study Procedure Calendar

	Screening Visit 1	Screening Visit 2	Medication Visit	Study Day 1	Study Day 2	Study Day 3
Complete History and Physical Examination	√					
Baseline Blood Pressure Measurements	√		√	√	√	√
Height, Weight	√			√	√	√
Hips and Waist Circumference	√					
CBC, CMP, lipid profile, urinalysis, 12-Lead ECG, HbA1C	√					
Oral Glucose Tolerance Test		√				
Urine Human Chorionic Gonadotrophin (β-HCG)		√		√	√	√
Urinary Sodium, potassium, and catecholamine Excretion				√	√	√
Power Spectral analysis				√	√	√
Serial Blood Pressure and Heart Rate Measurements				√	√	√
Plasmatic substance P, NPY, DPP-4 activity, ACE activity				√	√	√
PRA, Aldosterone, Angiotensin II, Plasma Catecholamines, tPA				√	√	√
Glucose, Insulin, Plasma Potassium				√	√	√
DNA	√					
Pill Dispensing			√	√	√	
Pill Count and Structured Pill taking Interview				√	√	√
Concurrent Conditions Interview				√	√	√
Adverse Event Checklist				√	√	√

REFERENCES

1. Ostchega Y, Yoon SS, Hughes J, Louis T. Hypertension awareness, treatment, and control-continued disparities in adults: United States, 2005-2006. http://www.cdc.gov/nchs/data/databriefs/db03.pdf. Accessed January 21, 2012.
2. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Prospective Studies Collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet. 2002 Dec 14;360(9349):1903–13.
3. Wang JG, Staessen JA, Franklin SS, Fagard R, Gueyffier F. Systolic and diastolic blood pressure lowering as determinants of cardiovascular outcome. Hypertension. 2005 May;45(5):907–13.
4. Chobanian AV, Bakris GL, Black HR, Cushman WC, et al. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. JAMA. 2003 May 21;289(19):2560–72.
5. Stamler J, Stamler R, Neaton JD. Blood pressure, systolic and diastolic, and cardiovascular risks. U.S. population data. Arch 4. Intern Med. 1993 Mar 8;153(5):598–615.
6. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction [see comments]. N Engl J Med 1998; 339(4):229-34.
7. Wingard DL, Barrett-Connor E: Heart disease and diabetes. In Diabetes in America. Washington, DC, U.S. Govt. Printing Office, 1995, p. 429-448 (NIH publ. no. 95-1468)
8. Horan, M. J.: Diabetes and hypertension. In Diabetes in America. Diabetes data compiled 1984. NIH Publication No. 85-1468. U.S. Government Printing Office, Washington, DC, 1985, ch. 17, pp. 1-22.
9. ADA. VI. Prevention, Management of Complications. Diabetes Care 2011;34(suppl 1):S31.
10. Chobanian A, Bakris G, Black H, Cushman W, Green L, Izzo J, Jones D, et al. Hypertension. 2003; 42: 1206-1252 JNC 7: Complete Report: Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension. 2003; 42: 1206-1252.
11. UK Prospective Diabetes Study Group: Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes (UKPDS 38). BMJ 317:703–713, 1998
12. Hansson L. The Hypertension Optimal Treatment study and the importance of lowering blood pressure. J Hypertens Suppl. 1999 Feb;17(1):S9-13.
13. Konzem SL, Devore VS, Bauer DW. Controlling hypertension in patients with diabetes. Am Fam Physician. 2002 Oct 1;66(7):1209-14.
14. National Kidney Foundation. Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. http://www.kidney.org/professionals/kdoqi/guideline_diabetes/guide3.htm. Accessed January 29, 2012.
15. World Health Organization, International Society of Hypertension Writing Group. 2003 WHO/International Society of Hypertension (ISH) Statement on Management of Hypertension. Journal of Hypertension 2003, 21: 1983-1992.

16. Kreymann B, Williams G, Ghatei M, Bloom S. Glucagon-like peptide-17–36: a physiological incretin in man. Lancet 2, 1300–1304 (1987).
17. Grouzmann E, Livio F, Buclin T. Angiotensin-Converting Enzyme and Dipeptidyl Peptidase IV Inhibitors: An Increased Risk of Angioedema. Hypertension. 2009;54:468-470.
18. Gorrell MD. Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. Clin Sci. 2005;108:277–292.
19. Mentlein R. Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. Regul Pept. 1999;85:9 –24.
20. McIntosh CH, Demuth HU, Pospisilik JA, Pederson R. Dipeptidyl peptidase IV inhibitors: How do they work as new antidiabetic agents? Regul. Pept. 2005; 128: 59–65.
21. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet. 2005 Jan 15-21;365(9455):217-23.
22. International Diabetes Federation. Diabetes Atlas. 3rd Edition. 2006. Brussels, International Diabetes Federation.
23. Matsas R., Kenny A, Turner A. The metabolism of neuropeptides: The hydrolysis of peptides, including enkephalins, tachykinins and their analogues by endopeptidase-24.11. Biochem. J. 223: 433-440, 1984.
24. Jackson EK, Dubinion JH, Mi Z. Effects of dipeptidyl peptidase IV inhibition on arterial blood pressure. Clin Exp Pharmacol Physiol. 2008;35:29–34.
25. Ahmad S, Wang L, Ward PE. Dipeptidyl (amino) peptidaseIV and aminopeptidase M metabolize circulating substance P in vivo. J Pharmacol Exp Ther 1992;260:1257-61.
26. Campos MM, Calixto JB. Neurokinin mediation of edema and inflammation. Neuropeptides 2003; 34:314-322.
27. Brown, Byiers, Carr D, Maldonado M, Warner BA. DPP-4 inhibitor use associated with increased risk of ACE-inhibitor associated angioedema.
28. Newby D, Sciberras D, Ferro C, Gertz B, Sommerville D, Majumdar A, Lowry R, Webb D. Substance P-induced vasodilatation is mediated by the neurokinin type 1 receptor but does not contribute to basal vascular tone in man. Br J Clin Pharmacol 1999; 48:336–344.
29. Marney A, Kunchakarra S, Byrne L, Brown N. Interactive Hemodynamic Effects of Dipeptidyl Peptidase-IV Inhibition and Angiotensin-Converting Enzyme Inhibition in Humans. Hypertension. 2010;56:728-733.
30. Petty M, Reid J. The cardiovascular effects of centrally administered substance P in the anaesthetized rabbit. Eur J Pharmacol. 1982 Aug 13;82(1-2):9-14.
31. Unger T, Rascher W, Schuster C, Pavlovitch R, Schömig A, Dietz R, Ganten D. Central blood pressure effects of substance P and angiotensin II: role of the sympathetic nervous system and vasopressin. Eur J Pharmacol. 1981 Apr 24;71(1):33-42.
32. Unger T, Rockhold RW, Yukimura T, Rettig R, Ganten D. Blood pressure and heart rate responses to centrally administered substance P are increased in spontaneously hypertensive rats. Clin Sci (Lond). 1980 Dec;59 Suppl 6:299s-302s.
33. Dzurik MV, Diedrich A, Black B, Paranjape SY, Raj SR, Byrne DW, Robertson D. Endogenous substance P modulates human cardiovascular regulation at rest and during orthostatic load. J Appl Physiol. 2007 Jun;102(6):2092-7.
34. Buck, SH. The tachykinin receptors. Clifton, NJ: Humana Press Inc. 1994.

35. Gustafsson, L. E.; Persson, M. G.; Wei, S.; Wiklund, N. P.; Elias, Y. Neurogenic vasodilation in rabbit hindlimb mediated by tachykinins and nitric oxide. J. Cardiovasc. Pharmacol. 23:612-617; 1994. 6.
36. Hall, J. M.; Brain, S. D. Inhibition by SR140333 of NK, tachykinin receptor-evoked, nitric oxide-dependent vasodilation in the hamster cheek pouch microvasculature in vivo. Br. J. Pharmacol. 113:522-526; 1994.
37. Boscan P, Kasparov S, Paton J. Somatic nociception activates NK1 receptors in the nucleus tractus solitarii to attenuate the baroreceptor cardiac reflex. Eur J Neurosci 2002; 16: 907-920.
38. Comet M, Laguzzi R, Hamon M, Sevoz-Couche C. Functional interaction between nucleus tractus solitarus NK1 and 5-HT3 receptors in the inhibition of baroreflex in rats. Cardiovasc Res 2005; 65; 930-939.
39. Pickering A, Boscan P, Paton J. Nociception attenuates parasympathetic but not sympathetic baroreflex via NK1 receptors in the rat nucleus tractus solitarii. J Physiol 2003; 551:589-599.
40. Riley J, Lin L, Chianca D, Talman W. Ablation of NK1 receptors in the rat nucleus tractus solitarii blocks baroreflexes. Hypertension 2002; 40:823-826.
41. Scheneider B, Gantem D, Lang R, Unger T. The central pressor action of substance P are inhibited by GABA. Eur J Pharmacol 1986, 131:31-37.
42. Ku Y, Tan L, Li L, Ding X. Role of corticotropine releasing factor and substance P in response of nucleai controlling emotion and stress. Peptides 1998, 19: 677-682.
43. Ervin RB, Wang C-Y, Wright JD, Kennedy-Stephenson J. Dietary Intake of Selected Minerals for the United States Population: 1999–2000 Advance Data From Vital and Health Statistics, No. 341. Hyattsville, MD: National Center for Health Statistics; 2004.
44. Jones B, Kenward MG. Design and Analysis of Crossover Trials, 2nd Ed., Boca Raton, FL, CRC Press LLC, 2003, pp 155.
45. Jones B, Kenward MG. Design and Analysis of Crossover Trials, 2nd Ed., Boca Raton, FL, CRC Press LLC, 2003, pp 212-213.
46. Wallin BG, Fagius J. The sympathetic nervous system in man. Aspects derived from microelectrode recordings. Trends Pharmacol Sci 1986;63-7.
47. Pagani M, Montano N, Porta A, Malliani A, Abboud FM, Birkett C, Somers VK. Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. Circulation 1997 March 18;95(6):1441-8.
48. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996 March 1;93(5):1043-65.

Vanderbilt University Institutional Review Board
Informed Consent Document for Research

Principal Investigator: Nancy J. Brown, M.D. Version Date: 9/26/17
Study Title: Contribution of Substance P to Blood Pressure Regulation in the Setting of Dipeptidyl Peptidase IV and Angiotensin Converting Enzyme (ACE) Inhibition
Institution/Hospital: Vanderbilt University

This informed consent applies to adults with high blood pressure and type 2 diabetes.

Name of participant: _____ Age: _____

The following is given to you to tell you about this research study. Please read this form with care and ask any questions you may have about this study. Your questions will be answered. Also, you will be given a copy of this consent form.

You do not have to be in this research study. You can stop being in this study at any time. If we learn something new that may affect the risks or benefits of this study, you will be told so that you can decide whether or not you still want to be in this study. Your medical record will contain a note saying you are in a research study. Anyone you authorize to receive your medical record will also get this note.

1. What is the purpose of this study?

You are being asked to take part in this research study because you have high blood pressure and type 2 diabetes.

The purpose of this study is to learn how a drug given for diabetes may affect blood pressure in someone taking certain drugs that help treat high blood pressure. About 150 people will take part in this study.

2. What will happen and how long will you be in the study?

Screening visit 1
If you decide to take part in the study, you will come to the Vanderbilt Clinical Research Center (CRC). You will have a complete physical, including height, weight, and waist. We will ask you about your medical history. We will take your blood (about 4 teaspoons) to check that you have no problems to keep you from being in the study. If you agree, part of this blood will be used for DNA (genetic testing.)

If you qualify to be in the study, you will be asked to stop taking your blood pressure drugs for 3 weeks. If you take hydrochlorothiazide (HCTZ), you may continue taking it while in the study. If you take spironolactone (Aldactone), you will be asked to stop taking it for 4 weeks. While you are off your blood pressure drugs, we will ask you to check your blood pressure every 1 to 3 days for the first week and once a week after that with a blood pressure monitor we will give you. If your blood pressure gets too high (upper number 170 or more, lower number 110 or more), you will be taken out of the study and restarted on your regular medicine.

If you are already taking metformin, you will continue taking it. If you take other medications for your diabetes, we will ask you to stop taking them for at least 3 weeks before the study. We will ask you to check your blood sugar at home every 1-3 days for the first week and once a week after that. If your blood sugar gets too low (70 mg/dL or less) or too high (300mg/dL or more), we will take you out of the study and restart you on your regular medicine.

Screening visit 2
You will come to the CRC in the morning, having had nothing to eat or drink since midnight. If you are a woman who could become pregnant, we will ask for a urine sample for a pregnancy test. If you are pregnant, you will not be allowed to be in the study.

We will measure your blood pressure and place a small tube in your vein to draw blood. We will take blood from your arm. We will then give you some liquid containing a measured amount of glucose to drink. We will take your

blood again 30, 60, 90, and 120 minutes after you drink the liquid. The total amount of blood taken during the test day will be a little more than a tablespoon.

Medication visit
At least 3 weeks after the screening visit, we will ask you to come to the CRC to pick up your study medications (pre-treatment: ramipril, valsartan, or amlodipine; first of the 1-week concurrent medications: sitagliptin plus placebo, sitagliptin plus aprepitant, or placebo plus placebo), a medication diary, and a urine collecting jug. A placebo is an inactive substance. You will be given either ramipril, valsartan, or amlodipine as pre-treatment. Ramipril, amlodipine, and valsartan are medicines are commonly used to treat high blood pressure. Placebo is a pill with no active ingredients. You will not be able to tell which you are taking by looking at the pill. Which pill you will get will be decided at random, like the toss of a coin. Neither you nor the study doctors will know which drug you are taking, but the doctors can find out if there is a need. You will take the medication once a day for the next 15 weeks. You will complete the medication diary daily, writing down if you take any other medications while on the study medication.

If you are a woman who could become pregnant, we will ask for a urine sample for a pregnancy test. If you are pregnant, you will not be allowed to be in the study.

After you have been taking the medication for 4 weeks you would start taking one of the 1-week concurrent medications. The order in which you receive each of the 1-week medications will be decided at random. Sitagliptin is used to treat diabetes. Aprepitant is used to treat nausea and vomiting after chemotherapy. You will take the medication once a day for the week before your study day.

We will ask you not to drink any alcohol or caffeine for 1 week before the study day.

Starting at least 3 days before the study day, we will ask you not to exercise.

Starting the morning before your first study day, you will collect all the urine you produce and save it in the jug we gave you. Bring the urine samples when you come for your study day. We will ask that you not have anything to eat or drink after midnight.

Study Day 1
On the day of your study, you will come to the CRC. We will measure your blood pressure and heart rate. If you are a woman who could become pregnant, we will ask for a urine sample to do a pregnancy test. If you are pregnant, you will not be allowed to be in the study.

We will place a blood pressure cuff on one arm and a small plastic tube (catheter) in the vein of your other arm. We will place a small blood pressure cuff on one of your fingers, and will place sticky patches on your chest to record your electrocardiogram (ECG – a recording of your heart's electrical activity.) We will ask you to lie down and rest quietly for 10 minutes. We then then measure your blood pressure and heart rate continuously for 5 minutes. We will give you your study medication for the day with a small amount of water.

Your blood pressure and heart rate will be measured every 5 minutes for the next four hours. We will take blood from the tube in your arm once every hour for the next 4 hours.

You will then be asked to stand up for 30 minutes. We will record your blood pressure and heart rate every 5 minutes while you are standing. At the end of this time, we will take another blood sample from your arm.

After the last blood draw, you will be finished with this study day. We will give you a regular meal before you leave. We will give you the medications for the next study day, a medication diary, and urine jug to take with you when you leave.

The total amount of blood taken for this study day will be about 7 tablespoons.

The study nurse or a study doctor will call you the day after and one week after your study day to check how you are doing.

We will ask you not to drink any alcohol or caffeine for 1 week before the study day.

Starting the morning before your first study day, you will collect all the urine you produce and save it in the jug we gave you. Bring the urine samples when you come for your study day. We will ask that you not have anything to eat or drink after midnight.

Study Day 2
On the day of your study, you will come to the CRC. The same research procedures listed above under Study day 1 will be done.

We will give you the medications for the Study day 3, a medication diary, and urine jug to take with you when you leave.

The total amount of blood taken for this study day will be about 16 tablespoons.

The study nurse will call you the day after and one week after your study day to check how you are doing.

We will ask you not to drink any alcohol or caffeine for 1 week before the study day..

Starting the morning before Study Day 3, you will collect all the urine you produce and save it in the jug we gave you. Bring the urine samples when you come for your study day. We will ask that you not have anything to eat or drink after midnight.

Study Day 3
On the day of your study, you will come to the CRC. The same research procedures listed above under Study day 1 will be done.
93
After the last blood draw, you will be finished with this study. We will give you a regular meal before you leave.
The total amount of blood taken for this study day will be about 16 tablespoons.

Your samples and information about you may be made available to others to use for research. To protect your privacy, we will not release your name. You will not receive any benefit as a result of the tests done on your samples. These tests may help us learn more about the causes, risks, treatments, or how to prevent high blood pressure and other health problems.

Your samples may be used to make new products or tests. These may have value and may be developed and owned by the study staff, Vanderbilt University, and/or others. If this happens, there are no plans to provide money to you.

3. Costs to you if you take part in this study:

There is no cost to you for taking part in this study.

4. Side effects and risks that you can expect if you take part in this study:

Inconveniences

- Not eating or drinking after midnight on the night before each study day
- Collecting all your urine in a jug for 24 hours on three occasionsTaking study medication daily may not be convenient.
- Not exercising for at least 3 days before each study day.
- Not consuming caffeine for 1 week may cause a headache if you regularly consume caffeine.

Risks of the Catheters

Putting a catheter into your vein to draw blood may cause pain, redness, soreness, bruising, or infection at the needle stick site. Rarely some people faint. We will use careful and sterile techniques to minimize these side effects.

ECG Risks

The sticky pads used for the ECG may cause skin irritation.

Pregnancy Risks

The drugs used in this study may hurt an unborn child. If you take part in this study, you and any person you have sex with must use a barrier method of birth control, such as a diaphragm or condoms, while you are in this study. If you become pregnant while you are in this study, you must tell your doctor at once. Also, women must not breast feed while in this study. If you are a woman and are able to become pregnant, you will have a urine test to make sure that you are not pregnant before you receive treatment in this study.

Aprepitant Risks

The uncommon side effects among people who take aprepitant for nausea following surgery are constipation, low blood pressure, nausea, itching, fever, urinary tract infection, anemia (decrease in the blood's ability to carry oxygen and may cause fatigue, headaches, or shortness of breath), sleeplessness, headache, low heart rate, flatulence, and vomiting.

Aprepitant may cause birth control pills to be less effective. While you are in the study and for one complete month after you finish the study, you must use some other form of contraception. If you become pregnant while in the study, you should tell the study doctor.
There are many medications that may cause problems if taken with the study drug. Please tell the study doctor about all medications that you take. Please talk to the study doctor before taking any new prescription medications or any new over-the-counter medications while you are on the study.

Ramipril Risks

Common risks of ramipril include: cough, high blood potassium, and low blood pressure. Uncommon risks include: hoarseness, lightheadedness, excessive tiredness, weakness, headache, and dizziness. Rarely, ramipril may cause swelling of the face, throat, tongue, lips, eyes, hands, feet, ankles, or lower legs, difficulty breathing or swallowing, and fainting.

Valsartan Risks

Common risks of valsartan include diarrhea, high blood potassium, and low blood pressure. Uncommon risks include headache, dizziness, and excessive tiredness. Rarely, valsartan can cause fainting.

Sitagliptin Risks
Drugs like sitagliptin can cause lowered blood sugar (common). This can result in dizziness, nausea, shaking, sweating, fast heartbeat, vision changes, headache, anxiety, tiredness, or confusion. It can rarely cause fainting, seizures, or coma. Taking enalapril and sitagliptin together might cause a swelling of the lips, tongue, mouth, or face (uncommon). If severe, this swelling can cause breathing problems. Other side effects are pancreatitis (damage to the pancreas), kidney damage (which can be life-threatening), allergic reaction (which can be life-threatening), rash, and liver damage (which can be life-threatening).

Drugs like sitagliptin may also cause joint pain that can be severe and disabling. This pain goes away after the medication is stopped, usually in less than a month. If you develop joint pain, please call the study doctor immediately and stop taking the medication.

Amlodipine Risks
Common risks of amlodipine include edema (swelling), low blood pressure, muscle cramps, dizziness, fatigue, fast heartbeat, nausea, and abdominal pain. Uncommon risks include rash, low heart rate, yellowing of the skin or eyes, decreased sexual drive, increased blood sugar, and insomnia.

5. Risks that are not known:

There may be risks that we do not know about at this time.

6. Payment in case you are injured because of this research study:

If it is determined by Vanderbilt and the Investigator that an injury occurred as a direct result of the tests or treatments that are done for research, then you and/or your insurance will not have to pay for the cost of immediate medical care provided **at Vanderbilt** to treat the injury.

There are no plans for Vanderbilt to pay for the costs of any additional care. There are no plans for Vanderbilt to give you money for the injury.

7. Good effects that might result from this study:

a) The benefits to science and humankind that might result from this study. We may learn more about how drugs for diabetes and high blood pressure may interact in the body.
b) The benefits you might get from being in this study. None.

8. Other treatments you could get if you decide not to be in this study:

This is not a treatment study. You may choose not to be in the study.

9. Payments for your time spent taking part in this study or expenses:

If you complete the study, you will be paid $600. If you do not complete the study, you will be paid $200 for each of the three study days you complete. We may ask you for your Social Security number and address before you are compensated for taking part in this study. This amount may be taxable and will be reported to the Internal Revenue Service (IRS).

10. Reasons why the study doctor may take you out of this study:

You may be removed from this study without your consent if staying in the study would be harmful to you or you no longer meet the requirements of the study, or if the study is stopped. If you are removed from the study, you will be told the reason.

11. What will happen if you decide to stop being in this study?

If you decide to stop being part of the study, you should tell your study doctor.

12. Who to call for any questions or in case you are injured:

If you should have any questions about this research study or if you feel you have been hurt by being a part of this study, please feel free to contact the study nurse Caleb Darby at 615-936-3458 or Dr. Jafarian at 615-981-3198**.** If you cannot reach the research staff, please page the study doctor, Dr. Brown at 615-835-7267.

For additional information about giving consent or your rights as a person in this study, to discuss problems, concerns, and questions, or to offer input, please feel free to call the Vanderbilt University Institutional Review Board Office at (615) 322-2918 or toll free at (866) 224-8273.

13. Clinical Trials Registry.

A description of this clinical trial will be available on www.clinicaltrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

14. Confidentiality:

Any private information we have obtained from you will be kept in a locked cabinet in the study doctor's office or in a password-protected computer. Only Dr. Brown and members of her study team will have access to your identified information. Any information we share with other researchers or publish will not identify you and will not contain your name.

The sponsor and/or Vanderbilt may give or sell your health data, without identifiers, to others or use it for other research projects not listed in this form. The sponsor, Vanderbilt, Dr Brown, and her staff will comply with any and all laws regarding the privacy of such information. There are no plans to pay you for the use or transfer of this de-identified information.

15. Authorization to Use/Disclose Protected Health Information

All efforts, within reason, will be made to keep your protected health information (PHI) private. PHI is your health information that is, or has been gathered or kept by Vanderbilt as a result of your healthcare. This includes data gathered for research studies that can be traced back to you. Using or sharing ("disclosure") such data must follow federal privacy rules. By signing the consent for this study, you are agreeing ("authorization") to the uses and likely sharing of your PHI. If you decide to be in this research study, you are also agreeing to let the study team use and share your PHI as described below.

As part of the study, Dr. Brown and her study team may share the results of your study and/or non-study linked blood tests and ECGs, as well as parts of your medical record, to the groups named below. These groups may include people from the Federal Government Office for Human Research Protections, the Vanderbilt University Institutional Review Board, and the National Institutes of Health. Federal privacy rules may not apply to these groups; they have their own rules and codes to assure that all efforts, within reason, will be made to keep your PHI private.

The study results will be kept in your research record for at least six years after the study is finished. At that time, the research data that has not been put in your medical record will be kept for an unknown length of time. Any research data that has been put into your medical record will be kept for an unknown length of time.
Unless told otherwise, your consent to use or share your PHI does not expire. If you change your mind, we ask that you contact Dr. Brown in writing and let her know that you withdraw your consent. Her mailing address is

Dr. Nancy Brown
D 3100 Medical Center North
Department of Medicine
Vanderbilt University
Nashville, TN 37232-2358

At that time, we will stop getting any more data about you. But, the health data we stored before you withdrew your consent may still be used for reporting and research quality.

If you decide not to take part in this research study, it will not affect your treatment, payment or enrollment in any health plans or affect your ability to get benefits. You will get a copy of this form after it is signed.

STATEMENT BY PERSON AGREEING TO BE IN THIS STUDY

I have read this consent form and the research study has been explained to me verbally. All my questions have been answered, and I freely and voluntarily choose to take part in this study.

Date	Signature of patient/volunteer

Consent obtained by:

Date	Signature
	Printed Name and Title

Consent for Genetic Research

The purpose of this study is to look at genes (DNA) and how they affect health and disease. Genes are the instruction manual for your body. The genes you get from your parents decide what you look like and how your body behaves. They can also tell us a person's risk for certain diseases and how they will respond to treatment.

You are being asked to give a blood sample for genetic research. What we learn about you from this sample will not be put in your health record. Your test results will not be shared with you or your doctor. No one else (like a relative, boss, or insurance company) will be given your test results. Health insurance companies and group health plans may not request your genetic information that comes from this research.

A single blood sample of 2 teaspoons will be taken from your arm at the same time we are taking another sample. This will not take any extra time.

One risk of giving samples for this research may be the release of your name that could link you to the stored samples and/or the results of the tests run on your samples. This may cause problems with insurance or getting a job.

Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums. Employers with 15 or more employees may not use your genetic information that comes from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment.

To prevent this, these samples will be given a code. Only the study staff will know the code. The name that belongs to the code will be kept in a locked file or in a computer with a password. Only Dr. Brown and members of her study team will have access to your name.

Your sample will be used to make DNA that will be kept for an unknown length of time (maybe years) for future research. The sample will be destroyed when it is no longer needed.

Your samples and information about you may be shared with others to use for research. To protect your privacy, we will not release your name.

You will not receive any benefit as a result of the tests done on your samples. These tests may help us learn more about the causes, risks, treatments, or how to prevent this and other health problems.

Giving samples for research is your free choice and you may be in the study even if you do not want your samples used or stored for gene research.

At any time, you may ask to have your sample destroyed. You should contact

Dr. Nancy Brown
D 3100 Medical Center North
Department of Medicine
Vanderbilt University
Nashville, TN 37232-2358

to have your sample destroyed and no longer used for research. We will not be able to destroy research data that has already been gathered using your sample. Also, if your identity was removed from the samples, we will not be able to locate and destroy them.

There will be no costs to you for any of the tests done on your samples. You will not be paid for the use of your samples.

Please check Yes or No to the questions below:

My blood/tissue sample may be used for gene research.

☐ Yes ☐ No

My blood/tissue sample may be stored/shared for future gene research in diabetes and heart disease.

☐ Yes ☐ No

My blood/tissue sample may be stored/shared for future gene research for other health problems (such as cancer, arthritis, etc).

☐ Yes ☐ No

Signature: _____ Date: _____