Substance P Increases Sympathetic Activity During Combined Angiotensin-Converting Enzyme and Dipeptidyl Peptidase-4 Inhibition

Jessica K. Devin, Mias Pretorius, Hui Nian, Chang Yu, Frederic T. Billings IV, Nancy J. Brown

Abstract—Dipeptidyl peptidase-4 inhibitors prevent the degradation of incretin hormones and reduce postprandial hyperglycemia in patients with type 2 diabetes mellitus. Dipeptidyl peptidase-4 degrades other peptides with a penultimate proline or alanine, including bradykinin and substance P, which are also substrates of angiotensin-converting enzyme (ACE). During ACE inhibition, substance P is inactivated primarily by dipeptidyl peptidase-4, whereas bradykinin is first inactivated by aminopeptidase P. This study tested the hypothesis that dipeptidyl peptidase-4 inhibition potentiates vasodilator and fibrinolytic responses to substance P when ACE is inhibited. Twelve healthy subjects participated in this randomized, double-blinded, placebo-controlled crossover study. On each study day, subjects received sitagliptin 200 mg by mouth or placebo. Substance P and bradykinin were infused via brachial artery before and during intra-arterial enalaprilat. Sitagliptin and enalaprilat each reduced forearm vascular resistance and increased forearm blood flow without affecting mean arterial pressure, but there was no interactive effect of the inhibitors. Enalaprilat increased bradykinin-stimulated vasodilation and tissue plasminogen activator release; sitagliptin did not affect these responses to bradykinin. The vasodilator response to substance P was unaffected by sitagliptin and enalaprilat; however, substance P increased heart rate and vascular release of norepinephrine during combined ACE and dipeptidyl peptidase-4 inhibition. In women, sitagliptin diminished tissue plasminogen activator release in response to substance P both alone and during enalaprilat. Substance P increases sympathetic activity during combined ACE and dipeptidyl peptidase-4 inhibition.

Clinical Trial Registration:—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01413542. (Hypertension. 2014;63:951-957.) • Online Data Supplement

Key Words: diabetes mellitus ■ dipeptidyl peptidase-4 ■ hypertension ■ norepinephrine ■ renin-angiotensin system ■ vasodilation

Dipeptidyl peptidase-4 (DPP4) is a ubiquitously expressed cell surface protease that preferentially cleaves dipeptides from the amino terminus of peptides containing a penultimate alanine or proline. A soluble form of DPP4 also exists in plasma.1 The first selective DPP4 inhibitor, sitagliptin, was approved by the Food and Drug Administration in 2006 for the management of hyperglycemia in patients with type 2 diabetes mellitus (T2DM). DPP4 inhibition decreases the degradation of endogenous incretin hormones, including glucagon-like peptide 1 (GLP-1), and thereby augments nutrient-mediated insulin release, suppresses glucagon secretion, and slows gastric emptying.2

The widespread expression of DPP4 within the vasculature and immune system raises the possibility that DPP4 could affect vascular function.3 Among the vasoactive substrates cleaved by DPP4 are the angiotensin-converting enzyme (ACE) substrates bradykinin and substance P. ACE inactivates these peptides by cleaving them at the carboxy terminus. However, when ACE is inhibited, substance P is inactivated by DPP4,4,5 whereas bradykinin is inactivated primarily by aminopeptidase P before it is cleaved by DPP4.6 Substance P and bradykinin are potent vasodilators and enhance endothelial fibrinolytic function by stimulating the release of tissue plasminogen activator (tPA).7,8 Substance P released from primary afferent sensory nerve fibers also increases sympathetic activity.9–11

ACE inhibitors are widely prescribed to patients with T2DM to reduce cardiovascular risk.12 Thus, many patients are treated concurrently with an ACE inhibitor and DPP4 inhibitor. This study tested the hypothesis that DPP4 inhibition potentiates the vasodilator, fibrinolytic, and sympathetic responses to substance P in the human forearm vasculature in the presence of ACE inhibition. Because bradykinin is inactivated by aminopeptidase P before cleavage by DPP4,6 we anticipated that ACE inhibition would potentiate the vasodilator response to bradykinin, but that DPP4 inhibition would not alter this effect.
Methods

Study Protocol

Twelve healthy, nonobese (body mass index <30 kg/m²), non-smoking adults participated in a double-blinded, randomized, placebo-controlled, crossover study (Figure 1; See Table S1 in the online-only Data Supplement for subject characteristics.) The study adhered to the principles of the Declaration of Helsinki and Title 45, US Code of Federal Regulations, Part 46, Protection of Human Subjects after approval by the Vanderbilt University Institutional Review Board, and all subjects provided written informed consent before initiation of study procedures. Patients with a history of chronic illness, including diabetes mellitus, hypertension, cardiovascular disease, and chronic renal or hepatic insufficiency, were excluded from participation. Medication use other than a multivitamin was prohibited at the time of study. Pregnancy was excluded in women of child-bearing age.

Each subject was studied on 2 days ≥1 week apart. Subjects were assigned to treatment order (sitagliptin or matching placebo) using a block randomization algorithm provided by the study biostatistician. Randomization was stratified by race and sex. On each study day, subjects reported to the Vanderbilt Clinical Research Center in the morning after an overnight fast. All subjects were studied in the supine position in a temperature-controlled room. Subjects were given oral study drug (sitagliptin 200 mg or matching placebo), and an arterial line was placed in the brachial artery of their nondominant arm with an adjacent peripheral intravenous line.

Baseline forearm blood flow (FBF) measurements and blood sampling were obtained 60 minutes after ingestion of study drug and ≥30 minutes after insertion of arterial catheter. (See online supplement for information on study procedures and medications.) Subjects then received sequential intra-arterial infusions of substance P and bradykinin in random order. Each peptide was infused in 3 graded doses for 5 minutes each. FBF was assessed during the last 2 minutes of each dose, and arterial and venous blood samples were then drawn simultaneously. A 30-minute washout separated each infusion. This sequence was then repeated during ACE inhibition by intra-arterial enalaprilat. On the second study day, the protocol was repeated using the opposite study drug (sitagliptin or matching placebo). Blood pressure and heart rate were continuously monitored throughout each study day.

Statistical Analysis

Data are presented as mean±SD, unless otherwise noted. Potential carryover and period effects were tested by comparing the measures of FBF obtained before each infusion. Mixed-effect models were used to analyze the data with a random subject effect and with fixed effects of treatment (enalaprilat and sitagliptin), vasodilator dose, as well as their interaction. Interaction terms were removed from the final model when the P value from the corresponding chunk test was >0.2. For inferences of interest, a 2-sided P value <0.05 was considered significant. Statistical analyses were performed using IBM SPSS software v. 21.0, GraphPad Prism 5, and R 2.15.0 (www.r-project.org).

Results

Effect of Treatment on DPP4 Activity and Baseline Hemodynamic Parameters

DPP4 inhibition with sitagliptin significantly decreased DPP4 activity compared with placebo (P=0.003), whereas DPP4 antigen was unchanged (Table). ACE inhibition significantly decreased ACE activity both in the presence (P=0.008) or absence of DPP4 inhibitor (P=0.01). Neither DPP4 inhibition nor ACE inhibition, alone or in combination, significantly affected FBF. ACE inhibition significantly decreased baseline forearm vascular resistance (P=0.04), as did DPP4 inhibition (P=0.01; Table). Similarly, ACE inhibition (P=0.04) and DPP4 inhibition (P=0.03) each increased FBF. DPP4 inhibition did not alter the effect of ACE inhibition on forearm vascular resistance or FBF at baseline.

Influence of DPP4 and ACE Inhibition on FBF, Heart Rate, and Norepinephrine Release

Vasodilator response is presented as FBF because local intra-arterial infusion of bradykinin or substance P did not affect mean arterial pressure in any treatment group. Intra-arterial bradykinin increased FBF in a dose-dependent manner (P<0.001), and ACE inhibition potentiated this effect (P<0.001; Figure 2). Treatment with DPP4 inhibition did not affect the vasodilator response to bradykinin. ACE inhibition significantly increased venous bradykinin concentrations (P<0.001) and decreased the metabolite bradykinin (1–5) (P>0.001); DPP4 inhibition did not affect bradykinin concentrations (data not shown). Intra-arterial substance P increased FBF in a dose-dependent manner (P<0.001); however, neither ACE inhibition nor DPP4 inhibition affected the vasodilator response to substance P.

Bradykinin did not affect heart rate either in the presence or in the absence of DPP4 and ACE inhibition (Figure 3). Substance P increased heart rate during ACE inhibition (from 61.2±8.8–65.7±6.8 bpm at the maximum dose of substance P, P=0.01) and during combined ACE and DPP4 inhibition (from 61.2±8.8–68.2±12.1 bpm at the maximum dose of substance P, P=0.03). Substance P-stimulated heart rate was also significantly higher during combined ACE and DPP4 inhibition than

![Figure 1. Study protocol. Each intra-arterial peptide was given in a randomized fashion as 3 graded doses, lasting 5 minutes each. A 30-minute washout separated infusions. Forearm blood flow measurement, followed by arterial and venous sampling, was performed at baseline and at completion of each dose of peptide. A minimum of 1 week separated each study day.](image-url)
during DPP4 inhibition alone (68.2 ± 12.1 versus 63.5 ± 11.3 bpm, P = 0.045).

At the highest dose of bradykinin, the arterial-venous (AV) norepinephrine gradient was similar during ACE, DPP4, and combined inhibition. Substance P did not significantly affect venous norepinephrine concentrations during ACE or DPP4 inhibition alone as compared with placebo. In contrast, during combined ACE inhibition and DPP4 inhibition, substance P increased venous norepinephrine (from 212.5 ± 60.7 during placebo to 331.9 ± 228.5 pg/mL at the highest dose during combined inhibition, P = 0.02) to a greater extent than arterial norepinephrine (206.6 ± 42.5 to 277.9 ± 126.0 pg/mL, P = 0.04). As a result, substance P increased the AV gradient of norepinephrine in the setting of combined DPP4 and ACE inhibition as compared with treatment with placebo (P = 0.05), ACE inhibition alone (P < 0.001, both men and women), and DPP4 inhibition alone (P = 0.04). Similarly, net norepinephrine release in response to substance P was significantly higher after treatment with combined DPP4 and ACE inhibition as compared with treatment with placebo (estimated difference 418.8 pg/min per 100 mL, 95% confidence intervals [93.9–743.8]; P = 0.01), ACE inhibition alone (estimated difference 534.1 pg/min per 100 mL, 95% confidence intervals [209.1–859.1]; P = 0.001), or DPP4 inhibition alone (estimated difference 447.2 pg/min per 100 mL, 95% confidence intervals [123.0–771.5]; P = 0.007). There was no effect of substance P on arterial or venous tryptase concentrations (data not shown).

Effect of DPP4 Inhibition on tPA Release in Response to Intra-Arterial Bradykinin and Substance P

During placebo, bradykinin increased net tPA release (P < 0.001) in a dose-dependent manner (Figure 4). ACE inhibition potentiated bradykinin-stimulated tPA release compared with placebo, in the absence of DPP4 inhibition (at the maximum bradykinin dose P < 0.001, both men and women) and in the presence of DPP4 inhibition (at maximum bradykinin dose P > 0.001, both men and women). As we have observed previously, the effect of ACE inhibition on bradykinin-stimulated tPA release was more pronounced in women. DPP4 inhibition did not influence bradykinin-stimulated tPA release. Substance P increased net tPA release (P < 0.001) in a dose-dependent manner during placebo (Figure 4 and Table S2). In women, DPP4 inhibition attenuated net tPA release in response to substance P as compared with placebo (P = 0.02 at maximum substance P dose). ACE inhibition potentiated substance P–stimulated tPA release in women but not in men (at maximum substance P dose P > 0.001 in women); this effect was attenuated by DPP4 inhibition (at maximum substance P dose P > 0.001 in women), such that the effect of ACE and DPP4 inhibition combined was no longer significant compared with placebo.

Safety

One woman experienced angioedema of the instrumented forearm after infusion of bradykinin and substance P on the sitagliptin treatment day. An upper extremity ultrasound did not show a deep vein thrombosis nor arterial thrombosis and the swelling resolved within 72 hours without intervention. Other adverse events included transient lightheadedness and

Table. Baseline Parameters

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placebo (n=12)</th>
<th>DPP4 Inhibition (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>ACE Inhibition</td>
</tr>
<tr>
<td>DPP4 activity, U/L</td>
<td>24.3±7.0</td>
<td>4.8±3.0*</td>
</tr>
<tr>
<td>DPP4 antigen, ng/mL</td>
<td>571.5±187.0</td>
<td>561.7±116.4</td>
</tr>
<tr>
<td>ACE act, U/L</td>
<td>37.4±7.6</td>
<td>8.1±3.3†</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>86.4±6.2</td>
<td>84.2±5.2</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>61.5±9.7</td>
<td>62.4±8.3</td>
</tr>
<tr>
<td>FVR, mm Hg/mL per min per 100 mL</td>
<td>37.7±12.4</td>
<td>30.4±10.0†</td>
</tr>
<tr>
<td>FBF, ml/min per 100 mL</td>
<td>2.6±0.8</td>
<td>3.2±1.1†</td>
</tr>
</tbody>
</table>

Results are presented as mean±SD.
ACE indicates angiotensin-converting enzyme; DPP4, dipeptidyl peptidase-4; FBF, forearm blood flow; FVR, forearm vascular resistance; and MAP, mean arterial pressure.

* P < 0.05 vs placebo.
† P < 0.05 vs placebo/vehicle.
‡ P < 0.05 vs DPP4 inhibition/vehicle.

Figure 2. Effect of treatment on forearm blood flow (FBF) response to intra-arterial bradykinin, with and without intra-arterial enalaprilat, and to substance P, with and without intra-arterial enalaprilat (n=12). Data presented as mean±standard error of the mean. Quadratic model–based P values presented in the text. P < 0.05 vs placebo at same peptide dose, † P < 0.05 vs dipeptidyl peptidase-4 inhibition (DPP4i) at same peptide dose. ACEi indicates angiotensin-converting enzyme inhibition.
orthostasis, which resolved with increased oral fluid intake (n=3) and 1 episode of nephrolithiasis within 2 weeks of completion of the first study day (n=1). There were no instances of hypoglycemia. One subject did not complete the second study day because of inability to obtain adequate arterial access; the data presented include this subject. All other subjects completed both study days.

Discussion

This study tested the hypothesis that DPP4 inhibition potentiates the vascular effects of substance P during ACE inhibition. We found no effect of combined DPP4 and ACE inhibition on substance P–mediated vasodilation; however, during concurrent DPP4 and ACE inhibition, intra-arterial administration of substance P stimulated the sympathetic nervous system. In addition, DPP4 inhibition diminished substance P–induced tPA release in women.

This study extends the findings of 3 previous studies examining the interactive effect of ACE inhibition and DPP4 inhibition on blood pressure. Marney et al\textsuperscript{14} reported that the DPP4 inhibitor sitagliptin reduced blood pressure in subjects with the metabolic syndrome when given alone for 5 days. When given in combination with an ACE inhibitor, however, sitagliptin attenuated the hypotensive response to ACE inhibition and caused an increase in heart rate and circulating norepinephrine concentrations.\textsuperscript{14} Boschmann et al\textsuperscript{15} reported a significant increase in postprandial venous norepinephrine after a 7-day treatment with the DPP4 inhibitor vildagliptin in patients with T2DM. The authors did not comment on concurrent ACE inhibitor use and hypothesized that the increased sympathetic activity may be a result of GLP-1 receptor activation in the central nervous system, as has been demonstrated in animal models.\textsuperscript{16,17} Jackson et al\textsuperscript{18} similarly reported that DPP4 inhibition increased blood pressure in spontaneously hypertensive rats only if they had been pretreated with an ACE inhibitor, and this effect was prevented by ganglionic blockade. The investigators postulated that decreased degradation of the vasoconstrictor neuropeptide Y by DPP4 may have contributed to increased blood pressure.\textsuperscript{18} The current study suggests, however, that activation of the sympathetic nervous system in the setting of combined ACE and DPP4 inhibition is in part mediated by substance P.

Substance P is a tachykinin neuropeptide, which is released fromafferent sensory nerve terminals and binds with highest affinity to the neurokinin-1 receptor.\textsuperscript{19} Nerve fibers containing substance P localize to the vasculature.\textsuperscript{20–22} Substance P–mediated vasodilation in the human forearm is dependent on activation of the neurokinin-1 receptor.\textsuperscript{21} Afferent sensory nerve stimulation or direct application of substance P results in depolarization of sympathetic neurons via the neurokinin-1 receptor.\textsuperscript{10,11,24} Substance P also stimulates release of catecholamines from the adrenal medulla in animal models\textsuperscript{10,25} and activates mast cells leading to increased formation of angiotensin II via mast cell–derived chymases.\textsuperscript{26} During concurrent DPP4 and ACE inhibition, substance P could increase heart rate and norepinephrine directly by stimulating sympathetic ganglia or indirectly by causing systemic vasodilation and reflex activation of the sympathetic nervous system. Two lines of evidence suggest that substance P facilitated local release of norepinephrine when ACE and DPP4 were inhibited. First, intra-arterial substance P increased the AV gradient during combined ACE and DPP4 inhibition, indicating local release. Second, intra-arterial infusion of substance P did not lower blood pressure even when both DPP4 and ACE were inhibited. On the contrary, the increase in heart rate observed during the highest dose of substance P during combined ACE and DPP4 inhibition suggests some degree of systemic sympathetic activation.

Increased sympathetic activation may also have contributed to the apparent decrease in substance P–stimulated tPA release in women during DPP4 inhibition. Net tPA release depends on both the AV gradient and blood flow and reflects the capacity of the vascular endothelium to release stored tPA. Norepinephrine, like substance P, stimulates tPA release from

![Figure 3](https://hyper.ahajournals.org/)

**Figure 3.** Effect of the maximal dose of substance P and bradykinin on heart rate (A) and norepinephrine release (B, C) after treatment with placebo, angiotensin-converting enzyme inhibitor (ACEi) alone, dipeptidyl peptidase-4 inhibitor (DPP4i) alone, or the combination (n=12). Data presented as mean±standard error of the mean. *P*<0.05 vs placebo, †*P*<0.05 vs DPP4i alone, ‡*P*<0.05 vs ACEi alone.
DPP4 inhibition decreases the degradation of histamine. We primarily result from the rapid release of histamine or other explanation is that substance P–mediated vasodilation may found no effect, however, of substance P on tryptase concentration. Additionally, enhanced sympathetic activation by substance P during combined ACE and DPP4 inhibition may result in norepinephrine-mediated vasoconstriction, thereby attenuating substance P–induced vasodilation. Finally, we may not have appreciated an effect of combined ACE and DPP4 inhibition on substance P–induced vasodilation because of tachyphylaxis, as has been previously observed during continuous infusion of substance P.

As previously described, one woman experienced edema of the instrumented forearm after receiving sitagliptin and subsequent intravenous infusions of bradykinin, substance P, and enalaprilat. Newby et al noted patchy skin edema, beginning at the level of the elbow and extending distally, in select healthy men receiving intra-arterial infusion of substance P at 16 pmol/min and in all subjects receiving 32 pmol/min of substance P. The maximum dose of substance P used in our protocol was 8 pmol/min, however, suggesting that the combination of ACE and DPP4 potentiated the effects of substance P on vascular permeability.

As noted earlier, this study provides mechanistic insight to previous studies examining the interactive effect of ACE and DPP4 inhibition on blood pressure. When given alone, DPP4 inhibitors have been reported to decrease blood pressure and to have conflicting effects on endothelial function. For example, 2 groups separately reported that sitagliptin or vildagliptin therapy reduced blood pressure. In animal models and in vitro, DPP4 inhibition lowers blood pressure and protects endothelial function, an effect that has been attributed to both GLP-1–dependent and GLP-1–independent increases in nitric oxide bioavailability. In patients with T2DM, DPP4 inhibition with vildagliptin potentiates endothelium-dependent vasodilation in response to acetylcholine. In contrast, Ayaori et al observed in 2 independent clinical trials that sitagliptin or alogliptin attenuate endothelial function as evaluated by flow-mediated vasodilation in patients with T2DM. We propose that interpretation of these disparate data on the vascular effects of DPP4 inhibitors in clinical trials may require knowledge of concurrent ACE inhibitor treatment.

Two recent placebo-controlled clinical trials, EXAMINE (EXamination of cardiovascular outcomes in patients with type 2 diabetes mellitus and acute coronary syndrome) and SAVOR-TIMI 53 (Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus-Thrombolysis in Myocardial Infarction 53), demonstrated that neither alogliptin nor saxagliptin affected the rate of cardiovascular events in patients with T2DM who were at high cardiovascular risk. The rate of hospitalization for heart failure, however, was increased with saxagliptin. Augmented sympathetic activity has long been implicated in the pathophysiology of heart failure. More than 50% of individuals taking saxagliptin were also prescribed an ACE inhibitor throughout the SAVOR-TIMI 53 trial. The contribution of a substance P–mediated increase in sympathetic activity to the development of heart failure in patients receiving concurrent DPP4 and ACE inhibition merits further study.

A few study limitations warrant mention. We studied the effect of acute DPP4 inhibition on the vasodilatory response to locally administered substance P and bradykinin with and without local ACE inhibition in the human forearm to isolate the vascular endothelium. Activation of the sympathetic nervous system during DPP4 inhibition may have caused systemic release of endothelial tPA, as evidenced by an increase in arterial tPA levels. Substance P infusion also stimulated local endothelial tPA release as supported by increased venous tPA levels. Stored endothelial tPA pools may have been depleted secondary to concurrent systemic tPA release mediated by the sympathetic nervous system and local tPA release mediated by substance P infusion, thus resulting in an apparent decrease in net tPA release.

Contrary to our hypothesis, ACE inhibition and DPP4 inhibition did not affect the vasodilator response to intra-arterial substance P. This is consistent with previous studies by Labinjoh et al and Benjamin and Webb that found no effect of acute or chronic ACE inhibition on substance P–stimulated arterial vasodilation but conflicts with studies demonstrating the hydrolysis of substance P by ACE and DPP4. One explanation is that substance P–mediated vasodilation may primarily result from the rapid release of histamine or other mediators from mast cells, and neither ACE inhibition nor DPP4 inhibition decreases the degradation of histamine. We found no effect, however, of substance P on tryptase concentration, a marker of mast cell activation. Alternatively, enhanced tPA release in response to intra-arterial bradykinin, with and without intra-arterial enalaprilat, and substance P, with and without intra-arterial enalaprilat (n=7 women, n=5 men). Data presented as mean±standard error of the mean. Linear model–based P values presented in the text. *P<0.05 vs placebo at same peptide dose, †P<0.05 vs dipeptidyl peptidase-4 inhibition (DPP4i) at same peptide dose, ‡P<0.05 vs angiotensin-converting enzyme inhibition (ACEi) at same peptide dose. Bradykinin and substance P increased tPA release in a dose-dependent manner (see text for P values).

Figure 4. Effect of treatment on net tissue plasminogen activator (tPA) release in response to intra-arterial bradykinin, with and without intra-arterial enalaprilat, and substance P, with and without intra-arterial enalaprilat. Newby et al noted patchy skin edema, beginning at the level of the elbow and extending distally, in select healthy men receiving intra-arterial infusion of substance P at 16 pmol/min and in all subjects receiving 32 pmol/min of substance P. The maximum dose of substance P used in our protocol was 8 pmol/min, however, suggesting that the combination of ACE and DPP4 potentiated the effects of substance P on vascular permeability.
the vascular responses to these specific peptide substrates and how these responses were altered by DPP4 inhibition and dual inhibition. We used 200 mg of sitagliptin because this dose inhibits DPP4 inhibition to the same extent as the clinically approved dose of 100 mg but within a shorter time period.46 We studied vascular function in healthy subjects to avoid the confounding effects of other medications and disease states on the endothelium. We also studied healthy individuals in the fasting state to control for the fluctuations in other vasoactive hormones, including GLP-1 and insulin, and to control for medications and diseases that may affect endothelial function. Substance P levels are both reported to be low in diabetic patients with coronary artery disease50 and high in obese individuals with diabetes mellitus.51 We did not measure substance P concentrations. Future studies using a neuropephin-1 receptor antagonist, aprepitant, are needed to investigate the specific contribution of endogenous substance P to changes in adrenergic tone during dual inhibition. Studies of prolonged ACE and DPP4 inhibition in individuals with T2DM will also be necessary to confirm the clinical relevance of these findings.

Perspective

Diabetes mellitus is associated with increased risk of heart attack and stroke. Although many anti–diabetes mellitus therapies reduce hyperglycemia, most do not reduce cardiovascular risk and some even increase risk. DPP4 inhibitor therapy may influence cardiovascular risk and blood pressure by affecting the degradation of vasoactive peptides, including substance P. Our findings indicate that substance P increases sympathetic activity in the presence of combined ACE and DPP4 inhibition. Elevated sympathetic activity and heart rate could contribute to the recently reported increased rate of hospitalization for heart failure in patients taking the DPP4 inhibitor saxagliptin in the SAVOR-TIMI 53 trial.40 More than half of the patients participating in this trial were also prescribed an ACE inhibitor. Further studies will be needed to determine whether decreased degradation of substance P during chronic concurrent ACE and DPP4 inhibition is associated with increased risk of heart failure. In the interim, physicians prescribing simultaneous DPP4 inhibitor and ACE inhibitor therapies in patients with T2DM should monitor for this undesirable, interactive hemodynamic effect.

Acknowledgments

We thank Stacy Gilbert, Delia Woods, and Carol Meisch for their recruitment of volunteers and nursing assistance. We thank Shouzuo Wei, Anthony Dematteo, Zuofei Wang, the Hormone Assay Core, and the Clinical Research Center Core Laboratory for their technical assistance.

Sources of Funding

This research was supported by the National Institutes of Health (NIH) grants RO1HL079184 and HL060906, and in part by Vanderbilt Clinical and Translational Science Awards grant UL1 TR000445 from National Center for Advancing Translational Sciences/NIMH (N.J. Brown). J.K. Devin was supported by the Vanderbilt Physician Scientist Development Award and NIH grant SP30GM092386. F.T. Billings was supported by K23GM102676.

Disclosures

None.

References


Novelty and Significance

What Is New?

- Decreased degradation of substance P during concurrent angiotensin-converting enzyme (ACE) and dipeptidyl peptidase-4 (DPP4) inhibition increases sympathetic activity.

- DPP4 inhibition in women decreases tissue plasminogen activator (tPA) release in response to substance P.

What Is Relevant?

- ACE inhibitors are widely prescribed to patients with type 2 diabetes mellitus to reduce cardiovascular risk, and DPP4 inhibitors are prescribed to control postprandial hyperglycemia.

- Increased sympathetic activity contributes to the development of insulin resistance, vascular dysfunction, hypertension, and heart failure.

- Reduced fibrinolytic capacity is associated with thrombotic events.

Summary

Substance P increases sympathetic activity during concurrent ACE and DPP4 inhibition in healthy subjects. DPP4 inhibition attenuates tPA release by substance P in healthy women. Further studies are needed to determine whether simultaneous prescription of these common therapies in patients with type 2 diabetes mellitus contributes to the risk of heart failure.
Substance P Increases Sympathetic Activity During Combined Angiotensin-Converting Enzyme and Dipeptidyl Peptidase-4 Inhibition

Jessica K. Devin, Mias Pretorius, Hui Nian, Chang Yu, Frederic T. Billings IV and Nancy J. Brown

*Hypertension*. 2014;63:951-957; originally published online February 10, 2014; doi: 10.1161/HYPERTENSIONAHA.113.02767

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/63/5/951

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2014/02/10/HYPERTENSIONAHA.113.02767.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Hypertension* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Hypertension* is online at:
http://hyper.ahajournals.org//subscriptions/
Online Supplement

Substance P increases Sympathetic Activity during Combined Angiotensin Converting Enzyme and Dipeptidyl Peptidase-4 Inhibition

Devin et al: ACE and DPP4 inhibition increases norepinephrine

Jessica K. Devin, MD MSCI1; Mias Pretorius, MB, ChB, MSCI2; Hui Nian, MS3;
Chang Yu, PhD3; Frederic T. Billings IV, MD MSCI2; Nancy J. Brown MD1

1Department of Medicine, Vanderbilt University Medical Center, Nashville, TN
2Department of Anesthesiology, Vanderbilt University Medical Center, Nashville, TN
3Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN

Address for Correspondence:
Jessica K. Devin, MD MSCI
Assistant Professor
Division of Diabetes, Endocrinology and Metabolism
7465 MRB4, 2213 Garland Ave
Nashville, TN 37232-0475
Tel: 615-936-1665
Fax: 615-936-1667
Jessica.devin@vanderbilt.edu
Methods

**Insertion of Arterial Line and Intra-arterial Administration of Study Drugs**

After subdermal administration of 1% lidocaine, a size 3 French catheter (Cook Inc., Bloomington, IN) was inserted into the brachial artery of the non-dominant arm for direct intra-arterial administration of peptides and arterial blood sampling. Arterial catheter patency was maintained by infusion of intravenous fluid (5% dextrose in water) at a rate of 1 mL/minute.

Bradykinin (Clinalfa Basic, Bachem, Germany) was infused at 23.6, 47.2, and 94.3 pmol/min. Substance P (Clinalfa Basic, Bachem, Germany) was infused at 2, 4, and 8 pmol/min. Enalaprilat was administered at 0.33 µg/min per 100 mL forearm volume. Drug concentrations in the infusate were adjusted to maintain an infusion volume of 1 mL/minute.

**Forearm Blood Flow Measurements**

Forearm blood flow (FBF) was measured using mercury-in-silastic strain-gauge plethysmography. The wrist was supported in a sling to raise the level of the forearm to above the level of the atrium, and a strain gauge was placed around the widest part of the forearm of the non-dominant hand. The strain gauge was connected to a plethysmograph (model EC-6, D.E. Hokanson; Issaquah, WA) connected to a chart recorder to record flow measurements. For each measurement, a cuff placed around the upper arm was inflated to 45 mmHg with a rapid cuff inflator (model E-20 rapid cuff inflator and AG 101 cuff inflator air source, Hokanson; Issaquah, WA) to occlude venous outflow from the extremity. The hand was excluded from the measurement of blood flow by inflation of a pediatric sphygmomanometer cuff to 200 mmHg around the wrist. Forearm blood flow (FBF) measurements were recorded for approximately seven seconds, and a minimum of 6 readings were analyzed using Non-Invasive Vascular Program (Hokanson NIVP3 version 5.40; Bellevue, WA) software to obtain each mean. Forearm vascular resistance (FVR) was calculated as mean arterial pressure divided by FBF.

Arterio-venous concentration gradients were calculated by subtracting the plasma level measured in the simultaneously collected venous (C_V) sample from that in the arterial (C_A) sample. Forearm plasma flow (FPF) was calculated from the FBF and hematocrit corrected for 1% trapped plasma. Thus, net release was calculated at each time point: Net release = (C_V-C_A) x [FBF x ((101-hematocrit)/100)].

**Laboratory Analyses**

Simultaneous arterial and venous samples were obtained from the infused arm at baseline and at completion of each dose of peptide. All samples were obtained after the first 3 mL of blood were discarded. Blood samples were collected on ice, centrifuged immediately and plasma stored at -80°C until time of assay. DPP4 antigen concentration was determined by ELISA (eBioscience, San Diego, CA). DPP4 activity was assayed by incubating sera with a colorimetric substrate, L-glycyl-L-prolyl p-nitroanilide, hydrochloride (Sigma), at 37°C. ACE activity was measured by LabCorp (Burlington, NC). TPA antigen levels were measured using the TriniLIZE tPA Antigen double antibody sandwich ELISA (Tcoag, Co. Wicklow, Ireland) on
blood collected in acidified citrate. Blood for catecholamine measurement was collected in pre-chilled tubes containing sodium heparin. Samples were measured by high-performance liquid chromatography with electrochemical detection. Tryptase levels were measured using the Human Tryptase ELISA (Kamiya Biomedical Company, Seattle, WA). Blood samples (3mL) for assessment of bradykinin concentrations were collected into 9 mL chilled ethanol to prevent degradation and artifactual kinin production. Samples were chilled on ice for 20 minutes, immediately centrifuged, and the supernatant stored at -80°C until analysis by liquid chromatography-tandem mass spectrometry, as previously described. Internal standards for bradykinin and bradykinin (1-5) labeled with 13C(6), 15N(4) on Arginine were obtained from New England Peptide, Inc. (Gardner, MA).
References


Table S1: Subject Characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n=12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.1 ± 12.0</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>9 (75%)</td>
</tr>
<tr>
<td>Black</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>7 (58%)</td>
</tr>
<tr>
<td>Male</td>
<td>5 (42%)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>67.8 ± 12.3</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>24.3 ± 3.2</td>
</tr>
<tr>
<td>Menopause Status</td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>5 (71%)</td>
</tr>
<tr>
<td>Menopausal</td>
<td>2 (29%)</td>
</tr>
</tbody>
</table>

Results are presented as mean ± standard deviation.
**Table S2. Effect of Substance P Dose on Tissue Plasminogen Activator (tPA) in Women (n=7)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle</th>
<th>ACEi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP 0</td>
<td>SP 2</td>
</tr>
<tr>
<td>Arterial tPA (ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>3.7±1.7</td>
<td>4.1±1.5</td>
</tr>
<tr>
<td>DPP4i</td>
<td>4.5±1.8</td>
<td>5.2±3.0</td>
</tr>
<tr>
<td>Venous tPA (ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>3.8±1.7</td>
<td>6.0±2.7*</td>
</tr>
<tr>
<td>DPP4i</td>
<td>4.2±1.7</td>
<td>6.8±6.4</td>
</tr>
<tr>
<td>AV gradient (ng/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>0.1±1.3</td>
<td>2.0±2.2*</td>
</tr>
<tr>
<td>DPP4i</td>
<td>-0.4±0.4</td>
<td>1.6±3.7</td>
</tr>
</tbody>
</table>

Results are presented as mean± standard deviation.

SP, substance P dose (pmol/min); tPA, tissue plasminogen activator antigen; AV, arterial-venous; ACEi, angiotensin-converting enzyme inhibition; DPP4i, dipeptidyl peptidase-4 inhibition

* p<0.05 versus baseline (SP0) during same treatment; †p<0.05 versus placebo at same dose