Effect of Dipeptidyl Peptidase 4 Inhibition on Arterial Blood Pressure is Context Dependent

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Abstract—Because the effects of dipeptidyl peptidase 4 (DPP4) inhibitors on blood pressure are controversial, we examined the long-term effects of sitagliptin (80 mg/kg per day) on blood pressure (radiotelemetry) in spontaneously hypertensive rats (SHR), Wistar–Kyoto rats, and Zucker Diabetic-Sprague Dawley rats (metabolic syndrome model). In SHR, chronic (3 weeks) sitagliptin significantly increased systolic, mean, and diastolic blood pressures by 10.3, 9.2, and 7.9 mm Hg, respectively, a response abolished by coadministration of BIBP3226 (2 mg/kg per day; selective Y1 receptor antagonist). Sitagliptin also significantly increased blood pressure in SHR treated with hydralazine (vasodilator; 25 mg/kg per day) or enalapril (angiotensin-converting enzyme inhibitor; 10 mg/kg per day). In Wistar–Kyoto rats, chronic sitagliptin significantly decreased systolic, mean, and diastolic blood pressures (−1.8, −1.1, and −0.4 mm Hg, respectively). In Zucker Diabetic-Sprague Dawley rats, chronic sitagliptin decreased systolic, mean, and diastolic blood pressures by −7.7, −5.8, and −4.3 mm Hg, respectively, and did not alter the antihypertensive effects of chronic enalapril. Because DPP4 inhibitors impair the metabolism of neuropeptide Y1–36 (NPY1–36; Y1 receptor agonist) and glucagon-like peptide (GLP)-1(7–36)NH2 (GLP-1 receptor agonist), we examined renovascular responses to NPY1–36 and GLP-1(7–36)NH2 in isolated perfused SHR and Zucker Diabetic-Sprague Dawley kidneys pretreated with norepinephrine (to induce basal tone). In Zucker Diabetic-Sprague Dawley kidneys, NPY1–36 and GLP-1(7–36)NH2 exerted little, if any, effect on renovascular tone. In contrast, in SHR kidneys, both NPY1–36 and GLP-1(7–36)NH2 elicited potent and efficacious vasoconstriction. In conclusion: (1) The effects of DPP4 inhibitors on blood pressure are context dependent; (2) The context-dependent effects of DPP4 inhibitors are due in part to differential renovascular responses to its most important substrates (NPY1–36 and GLP-1(7–36)NH2); (3) Y1 receptor antagonists may prevent the prohypertensive and possibly augment the antihypertensive effects of DPP4 inhibitors. (Hypertension. 2015;65:00-00.) ● Online Data Supplement

Key Words: dipeptidyl peptidase 4 ■ sitagliptin ■ spontaneously hypertensive rat ■ Wistar-Kyoto rat ■ Y1 receptor

Dipeptidyl peptidase 4 (DPP4) inhibitors are a novel class of drugs for managing type 2 diabetes mellitus. hemoglobin A1c, inhibitors reduce fasting blood glucose levels and hemoglobin A1c, yet seldom cause hypoglycemia or increase body weight. For this reason, DPP4 inhibitors are widely and increasingly used to treat type 2 diabetes mellitus.

Often patients with type 2 diabetes mellitus have the metabolic syndrome and are hypertensive; thus a large percentage of patients in whom DPP4 inhibitors are indicated also have hypertensive as a comorbidity. Consequently, it is critical to understand whether and how chronic administration of DPP4 inhibitors affects arterial blood pressure. Presently, the effect of DPP4 inhibitors on arterial blood pressure remains controversial with some investigators reporting that DPP4 inhibition lowers blood pressure, others reporting that DPP4 inhibition can raise blood pressure, and others reporting that DPP4 inhibition can augment or attenuate the antihypertensive effects of enalapril depending on the dose of enalapril. Importantly, still others report that DPP4 inhibitors have little or no effect on blood pressure. For example, Alter et al10 report that DPP4 inhibition does not affect arterial blood pressure in hypertensive diabetic endothelial nitric oxide synthase knockout mice, and Chaykovska et al11 report that DPP4 inhibition does not alter blood pressure in renovascular hypertensive rats. We hypothesize that the effect of DPP4 inhibition on arterial blood pressure is highly context dependent. This hypothesis is based on the knowledge that DPP4 metabolizes a large spectrum of biologically active peptides (>35), some of which decrease blood pressure and some of which increase blood pressure. For example, DPP4 inhibitors decrease the metabolism of endogenous glucagon-like peptide-1 (GLP-1) receptor (GLP-1R) agonists, such as the incretin hormone GLP-1(7–36)NH2, and GLP-1R agonists are antihypertensive.14 DPP4 inhibitors also impair the metabolism of endogenous Y1 receptor (Y1, R) agonists, such as neuropeptide Y1–36 (NPY1–36), and Y1, R agonists may be prohypertensive.15 Therefore, the net effects of DPP4 inhibitors on arterial blood pressure may well depend on the prevailing balance of a large number of...
peptides that affect the kidneys, vasculature, sympathoadrenal axis and brain; and this balance may in turn depend on factors, such as genetics, physiological state, method of blood pressure measurement, diet, coadministration of antihypertensive drugs, and time frame of blood pressure measurement. In addition, differential cell signaling by DPP4 substrates could also influence the net chronic effects of DPP4 inhibitors on arterial blood pressure.

In this study, we examined carefully the long-term effects of the DPP4 inhibitor sitagliptin on systolic, mean, and diastolic blood pressures (SBP, MABP, and DBP, respectively) under a highly controlled environment. Blood pressures were recorded by radiotelemetry and weekly averages comprising a total of 1008 measures per rat were used to increase the ability to detect even small (1 mmHg) long-term effects on blood pressures. Moreover, these experiments were conducted in animals with genetic hypertension (spontaneously hypertensive rats [SHR]), in normotensive animals (Wistar–Kyoto rats [WKY]) and in animals with the metabolic syndrome (Zucker Diabetic–Sprague Dawley [ZDSD] rats, a new animal model that captures better than any existing models the complex phenotype of the metabolic syndrome). Studies were conducted in both naive animals and animals chronically treated with antihypertensive drugs. The results of this study demonstrate unequivocally that sitagliptin can increase, have little effect on or decrease arterial blood pressure depending on the animal model. Moreover, we provide strong evidence that variability in the long-term effects of DPP4 inhibition on arterial blood pressure is, at least in part, because of differential renovascular responses to DPP4s most important substrates; that is, NPY_{1-36} and GLP-1(7-36)NH\_2.

Methods

Animals

SHR and WKY (male; 12–13 weeks) were purchased from Tacome Farms (Germantown, NY). Animals were allowed 2 weeks after arrival to the University of Pittsburgh’s animal facility before implanting radiotelemetry devices. Male ZDSD were obtained from PreClinOmics (Indianapolis, IN). These animals were 6 weeks at the time of arrival and were placed in quarantine for 9 weeks before being delivered to our telemetry facility. The animals were then allowed another 3 weeks of acclimation before implantation of telemetry devices. ZDSD were developed recently at PreClinOmics and have been inbred for >35 generations. Although ZDSD have a functional leptin pathway and do not have an SHR genetic background, ZDSD exhibit spontaneous hyperphagia, polygenic obesity, insulin resistance, glucose intolerance, diabetes mellitus, hypertension, and dyslipidemia and express elevated markers of cardiovascular disease and comorbidities associated with diabetes mellitus, including nephropathy, osteoporosis, and abnormal wound healing\textsuperscript{16,17} (http://www.preclinomics.com/genetic-models/zbsd-rat/). All procedures were approved by the University of Pittsburgh’s Institutional Animal Care and Use Committee.

Radiotelemetry

Rats were anesthetized briefly with 2% isoflurane, and the catheter from a model TA11PA—C40 radiotransmitter [Data Sciences International (DSI), St. Paul, MN] was inserted into the femoral artery and advanced into the abdominal aorta. The body of the radiotransmitter was secured in the peritoneal cavity and the incision was closed with wound clips, which were removed 1 week later. Animals were placed in individual polycarbonate cages (length: 15\textsuperscript{°} width: 10\textsuperscript{°}; height: 8\textsuperscript{°}) with environmental enrichment (small house and chew toy), and the cage was placed on a model RPC-1 DSI receiver connected to a DSI Exchange Matrix (with a DSI Ambient Pressure Reference model APR-1), which in turn directed the signal to a DELL OptiPlex computer running DSI Dataquest A.R.T. 4.3 (Silver) software. The radiotelemetry facility is housed in a dedicated room with limited entry by personnel and rigorous control of humidity, temperature, and lighting.

Isolated Perfused Rat Kidney

Rats were anesthetized with 90 mg/kg of thiobutabarbital (intraperitoneal injection), and the left kidney was isolated and perfused (single pass mode with constant flow of 5 mL/min) with a Hugo Sachs Elektronik-Harvard Apparatus GmbH (March—Hugstetten) perfusion system while perfusion pressure was monitored with a pressure transducer.

We have previously described this method in detail\textsuperscript{14} and have used this technique in many studies.\textsuperscript{16–22}

Analytic Measurements

Serum DPP4 activity was measured using the BioVision DPP4 Activity Fluorometric Assay Kit (Milpitas, CA), and the active forms of GLP-1 (GLP-1(7–36)NH\_2 and GLP-1(7–37)) in plasma were determined using IBL GLP-1 Assay Kit (Minneapolis, MN).

Drugs

Pharmaceutical grade sitagliptin and enalapril were from Merck (Whitehouse Station, NJ) and Mylan (Canonsburg, PA), respectively; hydralazine, norepinephrine, and thiobutabarbital were from Sigma-Aldrich (St. Louis, MO); BBP3226 and AMD3100 were from R&D Systems, Inc (Minneapolis, MN); and NPY\textsubscript{1-36} and GLP-1(7–36)NH\_2 were from ThermoFisher Scientific (Waltham, MA).

Statistical Analysis

Data were analyzed by 2-tailed Student\textit{t} tests or by repeated measures ANOVA with post hoc Fisher LSD tests (only if the overall ANOVA \textit{P}-value was significant.) The statistical package was Number Cruncher Statistical System 2004 (Kaysville, UT). The criterion for significance was \textit{P}<0.05.

Results

Chronic Effects of Sitagliptin on Arterial Blood Pressures in SHR

SHR (n=12) were instrumented with radiotransmitters and allowed a 3-week stabilization period before collecting basal blood pressures for 1 week. Average SBPs, MABPs, and DBPs during this week were 181±5, 159±2, and 138±3 mmHg, respectively. SHR were then treated chronically with hydralazine in the drinking water (25 mg/kg per day). The average weight at this time was ±350 g. The rationale for pretreating with hydralazine was that in our previously published studies acute DPP4 inhibition did not acutely increase MABP in naive SHR, but did so in SHR pretreated acutely with hydralazine.\textsuperscript{8} In this study, chronic hydralazine effectively decreased blood pressure such that during the second week of hydralazine administration the average SBPs, MABPs, and DBPs were 149±2, 128±2, and 110±3 mmHg, respectively. Although continuing hydralazine, sitagliptin was initiated at 80 mg/kg per day, a dose shown previously to inhibit DPP4 activity in rats by 85%.\textsuperscript{23} Sitagliptin treatment was continued for 3 weeks followed by a 2-week recovery period in which hydralazine was continued, but sitagliptin was discontinued. Repeated measures ANOVAs demonstrated highly significant differences in
SBPs ($P<0.0001$; Figure 1A), MABPs ($P<0.0001$; Figure 2A), and DBPs ($P=0.0019$; Figure 3A) across 6 weeks of observation (hydralazine only the week before initiating sitagliptin; sitagliptin+hydralazine for 3 weeks; and hydralazine only for 2 weeks after stopping sitagliptin). Post hoc tests showed that SBPs, MABPs, and DBPs were statistically significantly elevated during the 3 weeks of sitagliptin+hydralazine compared with either the week preceding sitagliptin (hydralazine only) or the 2 weeks post sitagliptin (again hydralazine only). The peak increases in SBP, MABP, and DBP occurred during the second week of sitagliptin, and these increases relative to the pre sitagliptin baseline were 3.7, 4.0, and 3.9 mm Hg, respectively, and relative to the post sitagliptin baseline (second week of recovery) were 6.3, 4.4, and 2.2 mm Hg, respectively.

To determine whether sitagliptin affects arterial blood pressures in SHR in the absence of antihypertensive drugs, the SHR were given a 2-week washout period to allow clearance of sitagliptin and hydralazine, and those animals that still had a normal pulse pressure, indicating that the radiotransmitters and catheters were still functioning normally ($n=9$), were continued in the experiment. During the second week of the wash-out period, the average SBPs, MABPs, and DBPs in these SHR were $173\pm5$, $150\pm3$, and $130\pm4$ mm Hg, respectively (not significantly different from the previous baselines before administering hydralazine). Next, these SHR were treated for 3 weeks with sitagliptin followed by a 3-week recovery period. On initiation of sitagliptin treatment, SBPs (Figure 1B), MABPs (Figure 2B), and DBPs (Figure 3B) gradually increased. In this regard, repeated measures ANOVAs demonstrated highly significant differences in SBPs ($P<0.0001$; Figure 1B), MABPs ($P<0.0001$; Figure 2B), and DBPs ($P=0.0019$; Figure 3B) across 7 weeks of observation (1 control week, 3 weeks of sitagliptin, and 3 weeks of recovery). During the second week of sitagliptin, SBPs, MABPs, and DBPs were statistically significantly elevated compared with the control week, and during the third week of sitagliptin, SBPs, MABPs, and DBPs were significantly elevated compared with either the control week or the past 2 recovery weeks. The peak increases in SBP, MABP, and DBP occurred during the third week of sitagliptin, and these increases relative to the pre sitagliptin baseline were $10.3$, $9.2$, and $7.9$ mm Hg, respectively, and relative to the post sitagliptin baseline (third week of recovery) were $5.3$, $5.7$, and $5.6$ mm Hg, respectively.

After, the third week of recovery from sitagliptin, those animals that still had a normal pulse pressure, indicating that the radiotransmitters and catheters were still functioning normally ($n=7$), were continued in the experiment by treating them with enalapril ($10$ mg/kg per day). The rationale for pretreating with enalapril was that angiotensin-converting enzyme inhibitors are frequently administered to patients taking antidiabetic drugs, and sitagliptin has been shown to attenuate the acute response to enalapril in humans. During the second week of enalapril, average SBPs, MABPs, and DBPs in these SHR were $153\pm6$, $130\pm2$, and $111\pm3$ mm Hg, respectively. Next

Figure 1. Bar graphs illustrate the effects of sitagliptin (80 mg/kg per day) on systolic blood pressure (SBP) in spontaneously hypertensive rats (SHR) pretreated with hydralazine (25 mg/kg per day; A), in SHR not pretreated with an antihypertensive drug (B), in SHR pretreated with enalapril (10 mg/kg per day; C), and in Wistar-Kyoto rats (D). SBP was obtained by radiotelemetry (measurements acquired every 10 minutes, 24 hours a day, and 7 days/wk) and weekly averages comprising a total of 1008 measures per rat are depicted as means±SEMs. Basal SBP refers to (A) SBP in the presence of hydralazine the week before starting sitagliptin; (B) and (D) SBP in the absence of antihypertensives the week before starting sitagliptin; and (C) SBP in the presence of enalapril the week before starting sitagliptin.
these SHR were treated for 3 weeks with sitagliptin followed by a 2-week recovery period while continuing the enalapril. On initiation of sitagliptin treatment, SBPs (Figure 1C), MABPs (Figure 2C), and DBPs (Figure 3C) increased. In this regard, repeated measures ANOVAs demonstrated highly significant differences in SBPs ($P<0.0001$; Figure 1C), MABPs ($P<0.0001$; Figure 2C), and DBPs ($P=0.0019$; Figure 3C) across 6 weeks of observation (enalapril only the week before initiating sitagliptin; sitagliptin-enalapril for 3 weeks; and enalapril only for 2 weeks after stopping sitagliptin). SBPs, MABPs, and DBPs were statistically significantly elevated during the second week of sitagliptin+enalapril compared with the week preceding sitagliptin (enalapril only), and SBPs, MABPs, and DBPs were elevated during all 3 sitagliptin+enalapril weeks compared with the 2 weeks post sitagliptin (again enalapril only). The sitagliptin-induced peak increases in SBP, MABP, and DBP relative to the pre sitagliptin baseline were 3.8, 3.9, and 4.1 mm Hg, respectively, and relative to the post sitagliptin baseline (second week of recovery) were 11.2, 8.9, and 6.6 mm Hg, respectively.

Our previously published studies demonstrate that the acute prohypertensive effects of DPP4 inhibitors in SHR are mediated by Y$_1$Rs, which are activated by the endogenous DPP4 substrates, NPY$_{1-36}$ and PYY$_{1-36}$. Therefore, we hypothesized that the chronic prohypertensive effects of sitagliptin in SHR are also mediated by Y$_1$Rs. To test this concept, 8 additional SHR were instrumented with radiotransmitters for chronic measurement of arterial blood pressures. SHR were then allowed a 2-week stabilization period before collecting basal blood pressures for 4 weeks. At this time, the average weights were $\approx 350$ g. Next, half of the SHR were administered daily subcutaneous injections of BIBP3226 (selective Y$_1$R antagonist) dissolved in saline and half were administered daily subcutaneous injections of saline only. The dose of BIBP3226 used in this study ($2$ mg/kg per day) is $\approx 20\times$ greater than that previously shown to block the vascular effects of NPY$_{1-36}$, and previous studies show that even an intravenous dose of BIBP3226 in the range used in this study does not have off-target effects on receptors for norepinephrine, angiotensin, endothelin, or vasopressin. These treatments were maintained for the duration of the experiment. After 2 weeks of BIBP3226 or saline-only pretreatment, all rats were treated with sitagliptin ($80$ mg/kg per day) for 6 weeks. In addition, in SHR receiving BIBP3226, after 3 weeks of BIBP3226, the animals were also treated with subcutaneous injections of AMD3100 for 3 weeks. AMD3100 is a selective antagonist for the CXC chemokine receptor 4 (CXCR4), a receptor activated by stromal-derived factor-1$\alpha$. Stromal-derived factor-1$\alpha$ is both a substrate for DPP4 and is implicated in the pathophysiology of some forms of hypertension. In this study, we used a dose of AMD3100 ($5$ mg/kg per day) that was $5\times$ greater than that previously shown to be efficacious.
In rats. As shown in Figure 4, daily subcutaneous injections of saline or BIBP3226 did not affect basal SBPs, MABPs, or DBPs. Repeated measures ANOVA demonstrated that in the animals treated with injections of saline only, sitagliptin increased SBP (Figure 4A; P < 0.0001), MABP (Figure 4C; P = 0.0003), and DBP (Figure 4E; P = 0.0199). In contrast, sitagliptin did not affect SBP (Figure 4B; P = 0.1151), MABP (Figure 4D; P = 0.9911), or DBP (Figure 4F; P = 0.9145) in SHR treated with BIBP3226. Adding AMD3100 to BIBP3226 did not exert additional effects on arterial blood pressures.

Chronic Effects of Sitagliptin on Arterial Blood Pressures in WKY

WKY (n=9) were instrumented with radiotransmitters for chronic measurement of arterial blood pressures and were allowed a 3-week stabilization period before collecting basal blood pressures for 1 week. Average SBPs, MABPs, and DBPs during this week were 127±1, 105±1, and 88±1 mm Hg, respectively. The average weight at this time was ≈375 g. Next, WKY were treated for 3 weeks with sitagliptin followed by a 3-week recovery period. On initiation of sitagliptin treatment, SBPs (Figure 1D), MABPs (Figure 2D), and DBPs (Figure 3D) slightly decreased. In this regard, repeated measures ANOVAs demonstrated highly significant differences in SBPs (P = 0.0001; Figure 1D), MABPs (P = 0.0001; Figure 2D), and DBPs (P = 0.0071; Figure 3D) across 7 weeks of observation (1 control week, 3 weeks of sitagliptin, and 3 weeks of recovery). During all 3 weeks of sitagliptin, SBPs, MABPs, and DBPs were statistically significantly reduced compared with the second and third recovery weeks; however, SBPs, MABPs, and DBPs were not significantly reduced compared with the pre sitagliptin baseline. The maximum changes in SBP, MABP, and DBP occurred during the third week of sitagliptin, and these changes relative to the pre sitagliptin basal values were −1.8, −1.1, and −0.4 mm Hg, respectively, and relative to the post sitagliptin baseline (third week of recovery) were −4.6, −4.8, and −4.6 mm Hg, respectively.

Chronic Effects of Sitagliptin on Arterial Blood Pressures in ZDSD

ZDSD (n=9) were instrumented with radiotransmitters for chronic measurement of arterial blood pressures and were allowed a 3-week stabilization period before collecting basal blood pressures for 1 week. Average SBPs, MABPs, and DBPs during this week were 145±2, 119±1, and 99±1 mm Hg, respectively. The average weight at this time was ≈520 g. Next, some ZDSD (n=4) were treated for 2 weeks with sitagliptin, whereas a control group (n=5) was not administered sitagliptin. In the control group, there were no significant changes during weeks 2 and 3 relative to the basal (week 1) in SBP (Figure 5A; P = 0.3982), MABP (Figure 5C; P = 0.9073), or DBP (Figure 5E; P = 0.3494). In contrast, sitagliptin decreased SBPs (Figure 5B), MABPs (Figure 5D), and DBPs (Figure 5F). In this regard, repeated measures
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ANOVAs demonstrated highly significant differences in SBPs ($P=0.0121$; Figure 5B) and MABPs ($P=0.0049$; Figure 5D) and a strong trend in DBPs ($P=0.0987$; Figure 5F) across the 3 weeks of observation (1 control week; 2 weeks of sitagliptin). The maximum changes in SBP, MABP, and DBP occurred during the second week of sitagliptin, and these changes relative to the pre sitagliptin basal values were −7.7, −5.8, and −4.3 mmHg, respectively.

In a separate study, we also investigated whether sitagliptin alters the antihypertensive effects of enalapril (10 mg/kg per day) in ZDSD. ZDSD (n=6) were instrumented with radio-transmitters for chronic measurement of arterial blood pressures and were allowed a 3-week stabilization period. Then 3 ZDSD were treated with sitagliptin for 1 week, whereas the other 3 did not receive sitagliptin. After 1 week, all ZDSD were treated with enalapril for 2 weeks, followed by 2 additional weeks without enalapril. In ZDSD not pretreated or cotreated with sitagliptin, enalapril significantly decreased SBP, MABP, and DBP (Figure 6A, 6C, and 6E, respectively). Similarly, in ZDSD pretreated and cotreated with sitagliptin, enalapril significantly decreased SBP, MABP, and DBP (Figure 6B, 6D, and 6F, respectively). Importantly, the ability of enalapril to decrease arterial blood pressures in ZDSD was not affected by the pretreatment/cotreatment with sitagliptin.

### Chronic Effects of Sitagliptin on Heart Rates in SHR, WKY, and ZDSD

In hydralazine-pretreated/cotreated and naive SHR, chronic sitagliptin slightly, but significantly ($P<0.0001$), increased heart rate in SHR (Figure S1A and S1B in the online-only Data Supplement). However, this response was not observed in enalapril-pretreated/cotreated SHR (Figure S1C) or in naive WKY (Figure S1D) or in ZDSD (Figure S2); in fact, sitagliptin significantly lowered heart rate in enalapril-pretreated SHR and in ZDSD and tended to do so in WKY.

### Effects of NPY$_{1-36}$ and GLP-1(7–36)amide on Renovascular Responses in SHR Versus ZDSD Kidneys

NPY$_{1-36}$ and GLP-1(7–36)amide signal via Y$_1$Rs and GLP-1Rs, respectively, and these receptors signal in part via adenylyl cyclase (Gi for Y$_1$Rs and Gs for GLP-1R). Previous studies in SHR kidneys demonstrate that renovascular responses to Gi-coupled$^{2,3}$ and Gs-coupled$^{35,36}$ receptors are dysfunctional in SHR kidneys leading to increased renal vasoconstriction. Because renal function is the prime determinant of long-term blood pressure, we examined whether the renovascular responses to NPY$_{1-36}$ and GLP-1(7–36)amide (most important substrates for DPP4) are different in SHR versus ZDSD.

![Figure 4. Bar graphs illustrate the effects of sitagliptin (80 mg/kg per day) on systolic blood pressure (SBP; A and B), mean arterial blood pressure (MABP; C and D), and diastolic blood pressure (DBP; E and F) in spontaneously hypertensive rats (SHR) without (A, C, and E) or with BIBP3226 (BIBP; 2 mg/kg per day; B, D, and F). AMD3100 (AMD; 5 mg/kg per day) was administered during the past 3 weeks in SHR receiving BIBP3226. SBP was obtained by radiotelemetry (measurements acquired every 10 minutes, 24 hours a day, and 7 days/wk) and weekly averages comprising a total of 1008 measures per rat are depicted as means±SEMs.](http://hyper.ahajournals.org/)

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In this regard, kidneys from naive SHR, naive ZDSD, sitagliptin-treated (for 3 weeks) SHR and sitagliptin-treated (for 3 weeks) ZDSD were isolated, perfused, and exposed to increasing concentrations of NPY1–36 and GLP-1(7–36)amide (1, 3, 10, and 30 nmol/L in the perfusate; order of NPY1–36 and GLP-1(7–36)amide was random, ie, crossover design). These experiments were performed both in kidneys without basal tone and in kidneys receiving an infusion of norepinephrine into the renal artery (final concentration of 100 nmol/L) to provide basal tone. Also, kidneys from animals treated chronically with sitagliptin were supplemented with 1 μmol/L of sitagliptin in the perfusate. As shown in Figures 7A, 7B, 8A, and 8B, in the absence of basal tone, neither NPY1–36 nor GLP-1(7–36)amide affected renal perfusion pressure (indicator for renal vasoconstriction in this constant flow system) regardless of strain or pretreatment with sitagliptin. However, the results were much different in kidneys provided basal tone with norepinephrine. In this regard, in SHR kidneys, both NPY1–36 and GLP-1(7–36)amide caused potent vasoconstriction (Figures 7C and 8C, respectively), and this response was even greater in sitagliptin-treated kidneys (Figures 7D and 8D, respectively). In contrast to SHR and regardless of sitagliptin treatment, ZDSD kidneys expressed a mild vasoconstrictive response to NPY1–36 (Figures 7C and 7D) and exhibited a mild vasodilatory response to GLP-1(7–36)amide (Figures 8C and 8D). Thus, the renovascular responses to DPP4 substrates in SHR were qualitatively and qualitatively different compared with ZDSD.

Effects of Chronic Sitagliptin Treatment on Serum DPP4 Activity and Plasma Levels of GLP-1(7–36)amide+GLP-1(7–37)

To verify that the dose of sitagliptin used in this study was adequate to block DPP4 and to alter DPP4 substrate levels, we measured serum DPP4 activity and plasma GLP-1(7–36)NH₂+GLP-1(7–37) levels in naive SHR and ZDSD and in SHR and ZDSD treated for 3 weeks with sitagliptin (80 mg/kg per day). GLP-1(7–36)NH₂ and GLP-1(7–37) are metabolized to GLP-1(9–36)NH₂ and GLP-1(9–37) so inhibition of DPP4 should be associated with an increase in GLP-1(7–36)NH₂+GLP-1(7–37) levels. Although NPY1–36 is metabolized to NPY3–36 by DPP4,
currently there is no assay that differentiates NPY_{1–36} from NPY_{3–36}. Because total NPY (NPY_{1–36} plus NPY_{3–36}) would be of limited value, we did not attempt to measure NPY levels. As shown in Figure S3, serum DPP4 activity was similar in SHR versus ZDSD and in both strains chronic sitagliptin inhibited serum DPP4 activity by \approx 70\%. As shown in Figure S4, plasma GLP-1(7–36)NH\textsubscript{2}/GLP-1(7–37) levels were similar in SHR versus ZDSD and in both strains chronic sitagliptin increased plasma GLP-1(7–36)NH\textsubscript{2}+GLP-1(7–37) levels by \approx 60\%.

**Discussion**

The purpose of this investigation was to test the concept that the net chronic effects of DPP4 inhibition on blood pressure depend on context, a hypothesis based on the fact that DPP4 metabolizes multiple biologically active peptides.\textsuperscript{12,13} This hypothesis is also supported by our previous observation that in SHR and WKY pretreated with hydralazine, acute administration of P32/98 (DPP4 inhibitor) increases and decreases, respectively, short-term levels of arterial blood pressure.\textsuperscript{8} Moreover, clinical studies also suggest that the effects of DPP4 inhibition may be context dependent. For example, studies by Marney et al\textsuperscript{9} show that in subjects with the metabolic syndrome, sitagliptin increases the acute hypotensive effects of low-dose enalapril, yet decreases the acute hypotensive response to high-dose enalapril. Also, despite the fact that DPP4 inhibitors increase the levels of endogenous GLP-1R
agonists, chronic administration of the direct GLP-1R agonist exenatide has a hypotensive effect in type 2 diabetes mellitus, whereas sitagliptin does not, suggesting the involvement of complex changes in vasoactive peptides with sitagliptin.

The unique aspect of this study is that it represents the most comprehensive and technologically precise preclinical evaluation published to date on the effects of chronic DPP4 inhibition on long-term blood pressure regulation. Our experiments were performed (1) using the gold standard methodology for blood pressure monitoring (radiotelemetry); (2) under highly controlled environmental conditions; (3) during a chronic time interval for both dosing and blood pressure monitoring; (4) using 3 different strains of rats; and (5) in the absence and presence of antihypertensives.
In this study, chronic administration of sitagliptin increased SBP, MABP, and DBP in adult SHR. This was observed during 4 separate trials, such as 3 weeks of sitagliptin with hydralazine; 3 weeks of sitagliptin with enalapril; 3 weeks of sitagliptin with no antihypertensives; and 6 weeks of sitagliptin with no antihypertensives. It is a matter of semantics as to whether one refers to the increase in blood pressure by DPP4 inhibition in the presence of antihypertensive drugs as reducing the antihypertensive effects of the antihypertensive or a prohypertensive effect of DPP4 inhibition in the presence of antihypertensives. Because these descriptions convey the same result, they are equivalent. Pacheco et al examined the effects of sitagliptin (80 mg/kg per day for 8 days) on blood pressure (direct arterial cannulation) on a smaller group (n=7) of adult SHR and did not detect significant changes in SBP or DBP. Also, Liu et al monitored blood pressures in a small group (n=4) of SHR by radiotelemetry and noted that sitagliptin (10 mg/kg per day for 14 days) caused a decrease in SBP between days 3 and 4 of treatment but did not alter MABP or DBP. Given the complexity of the pharmacology of DPP4 inhibitors, it is not surprising that different laboratories using different methods obtain contrasting results. Indeed, in this study, we would not have been able to detect the modest effect of sitagliptin to increase blood pressure in SHR without the aid of radiotelemetry and under ideal conditions and using a large number of SHR.

In aggregate, DPP4 inhibitors seem to have little effect on blood pressure in humans. For example, in patients with hypertension treated for 5 days with sitagliptin, blood pressure decreased by 2 mm Hg; and 26 weeks of sitagliptin in type 2 diabetic patients did not affect blood pressure. A plausible hypothesis to explain this is that some patients respond with an increase, some with a decrease and others with no change in blood pressure. This hypothesis is corroborated by the observations in this study that in WKY, sitagliptin has less effect on blood pressure, whereas in ZDS, a model of the metabolic syndrome, sitagliptin clearly lowers blood pressure. The antihypertensive effect of DPP4 inhibition in ZDS is in agreement with the reports by Ferreira et al and Aroor et al that inhibition of DPP4 lowers blood pressure in Zucker Diabetic Fatty rats and Zucker obese rats. Importantly, we also observe that in ZDS rats chronic DPP4 inhibition with sitagliptin does not alter the antihypertensive effects of chronic angiotensin-converting enzyme inhibition with enalapril. This indicates that DPP4 inhibitors can be used in diabetic patients also receiving angiotensin-converting enzyme inhibitors without compromising the long-term antihypertensive effects of the angiotensin-converting enzyme inhibitor.

Why does chronic DPP4 inhibition increase blood pressure in SHR yet does not in WKY or ZDS? In this study, we measured serum levels of DPP4 activity and plasma levels of GLP-1(7–36)amide in SHR versus ZDS and found that (1) serum DPP4 activity is similar in SHR versus ZDS; (2) serum DPP4 activity is similarly suppressed by sitagliptin in SHR versus ZDS; and (3) sitagliptin elevates GLP-1(7–36)amide similarly in SHR versus ZDS. These findings indicate that the differential blood pressure response to DPP4 inhibition in SHR versus ZDS is unrelated to the basal or treatment levels of DPP4 activity. In this study, we did not measure NPY levels because currently available assays do not distinguish NPY1–36 from NPY1–36, and measuring total NPY gives no information about basal or drug-induced changes in levels of NPY1–36.

We hypothesize that DPP4 inhibitors differentially influence long-term levels of arterial blood pressure in different rat strains because the biologically active peptides affected by DPP4 inhibitors signal differently in the kidneys of these strains. As mentioned above, NPY1–36 and GLP-1(7–36)amide signal via Y1Rs (Gi-coupled to adenylyl cyclase) and GLP-1Rs (Gs-coupled to adenylyl cyclase), respectively. Importantly, published studies show that signaling via Gi-coupled and Gs-coupled receptors is different in the renal vasculature of SHR kidneys (compared with kidneys from normotensive rats) leading to increased renal vasoconstriction in SHR.

This study shows that when renovascular tone is primed with exogenous norepinephrine, the renal vasculature of SHR vasoconstricts in response to the DPP4 substrates NPY1–36 and GLP-1(7–36)amide, with vasoconstriction being most pronounced with NPY1–36. In stark contrast, ZDS kidneys express only a mild vasoconstrictive response to NPY1–36, and actually dilate in response to GLP-1(7–36)amide. Thus, renovascular responses to DPP4 substrates in SHR are both qualitatively and qualitatively different compared with ZDS. As chronic renal vasoconstriction is well known to elevate long-term levels of arterial blood pressure, the present findings suggest that the differential effects of chronic DPP4 inhibition on long-term levels of arterial blood pressure are likely the result of differential signal transduction in the renal vasculature.

Although the hypertensive response to DPP4 inhibitors in SHR could be mediated, in part, by GLP-1Rs, we hypothesize that Y1Rs are mostly responsible. In support of this idea, not only do SHR kidneys have a greater vasoconstrictor response to NPY1–36, both young and adult SHR also have elevated vascular levels of NPY. This study shows that BIBP3226 blocks the chronic increase in blood pressure induced by sitagliptin in SHR, which further corroborates the hypothesis that Y1R activation mediates the increase in blood pressure induced by sitagliptin in SHR. Although stromal-derived factor-1α is a substrate for DPP4 and is implicated in the pathophysiology of some forms of hypertension, antagonism of the CXC chemokine receptor 4 does not seem to affect blood pressure in sitagliptin-treated SHR.

Does the role of Y1Rs in the pressor mechanism of chronic DPP4 inhibitors in SHR have clinical relevance? Some studies show a correlation between blood pressure and NPY levels and demonstrate higher NPY levels in patients with essential hypertension. Recent evidence suggests an association of polymorphisms in the NPY promoter with hypertension. Thus, it is likely that some patients have NPY-dependent elevations in blood pressure, and DPP4 inhibitors would likely increase blood pressure in such patients.

Perspective

This study demonstrates that DPP4 inhibition can increase, decrease, or not affect arterial blood pressure, depending on the animal model. We conclude that the effect of this class of drugs on arterial blood pressure is context dependent; and this is likely because of complex alterations in the levels of biologically active peptides that would predictably accompany...
inhibition of DPP4. With respect to hypertension, the implication is that some hypertensive patients may benefit from an antihypertensive action, whereas others may experience an adverse increase in blood pressure. Our results also suggest that the increase in blood pressure induced by DPP4 inhibition can be prevented by Y1R antagonists. Importantly, DPP4 inhibition increases hospitalization for heart failure and NPY levels are elevated in patients with heart failure. It is plausible, although untested here, that Y1R blockade could augment the antihypertensive effects of DPP4 inhibition and eliminate the increased risk of heart failure induced by DPP4 inhibition.

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Disclosures
None.

References


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THE EFFECT OF DIPEPTIDYL PEPTIDASE-4 INHIBITION ON ARTERIAL BLOOD PRESSURE IS CONTEXT DEPENDENT

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Figure S1: Bar graphs illustrate the effects of sitagliptin (80 mg/kg/day) on heart rate (HR) in SHR pretreated with hydralazine (25 mg/kg/day) (A), in SHR not pretreated with an antihypertensive drug (B), in SHR pretreated with enalapril (10 mg/kg/day) (C) and in WKY (D). HR was obtained by radiotelemetry (measurements acquired every 10 minutes, 24 hours a day, 7 days per week) and weekly averages comprising a total of 1008 measures per rat are depicted as means ± SEMs. "Basal" HR refers to: in panel A HR in the presence of hydralazine the week before starting sitagliptin; in panels B and D HR in the absence of antihypertensives the week before starting sitagliptin; and in panel C HR in the presence of enalapril the week before starting sitagliptin.
Figure S2: Bar graphs illustrate HR in ZDSD without (A) or with (B) sitagliptin (80 mg/kg/day). HR was obtained by radiotelemetry (measurements acquired every 10 minutes, 24 hours a day, 7 days per week) and weekly averages comprising a total of 1008 measures per rat are depicted as means ± SEMs.
Figure S3: Bar graphs illustrate the effects of sitagliptin (80 mg/kg/day for 3 weeks) on serum DPP4 activity in SHR (A) or ZDSD (B). Values are means ± SEMs, and p-values are from 2-tailed Student's t-test.
Figure S4: Bar graphs illustrate the effects of sitagliptin (80 mg/kg/day for 3 weeks) on plasma levels of active GLP-1 [GLP-1(7-36)NH2 plus GLP-1(7-37)] in SHR (A) or ZDSD (B). Values are means ± SEMs, and p-values are from 2-tailed Student's t-test.