Published in final edited form as:

Clin Exp Pharmacol Physiol. 2010 July ; 37(7): 689–691. doi:10.1111/j.1440-1681.2010.05389.x.

Sitagliptin Augments Angiotensin II-Induced Renal Vasoconstriction In Kidneys from Rats with the Metabolic Syndrome

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SUMMARY

1. Dipeptidyl peptidase IV (DPPIV) inhibitors enhance renovascular responses to angiotensin II (Ang) in spontaneously hypertensive rats (SHR) but not Wistar-Kyoto rats. Because DPPIV inhibitors are often used in the metabolic syndrome, it is important to determine whether in this setting DPPIV inhibition enhances renovascular responses to Ang.
2. Six-week-old Lean-ZSF1 rats (harbor SHR genes; do not have the metabolic syndrome; n=11) and Obese-ZSF1 rats (harbor SHR genes; express the metabolic syndrome; n=10) were obtained from Charles River (Wilmington, MA).
3. At 7 weeks of age, compared to Lean-ZSF1, Obese-ZSF1 demonstrated significant ($p<0.05$) increases in body weight (262 ± 8 versus 310 ± 13 g), plasma glucose (112 ± 4 versus 153 ± 9 mg/dl), hemoglobin A1c (4.7 ± 0.1 versus 5.8 ± 0.4 %), urinary glucose excretion (0.02 ± 0.003 versus 6.70 ± 1.80 g/kg body weight/24 hours) and urinary protein excretion (100 ± 7 versus 313 ± 77 mg/kg body weight/24 hours). Mean blood pressure was high (133 ± 7 mmHg) in both strains.
4. At 8 weeks of age, kidneys were isolated and perfused. In Lean-ZSF1, renovascular responses (changes in perfusion pressure) to physiological levels of Ang (0.1 nmol/L) were 3.4 ± 1.3 (n=5) and 18.2 ± 5.9 (n=6) mmHg in untreated versus sitagliptin-treated (1 μ mol/L) kidneys, respectively. In Obese-ZSF1, renovascular responses to Ang were 5.5 ± 1.3 (n=4) and 17.8 ± 8.2 (n=6) mmHg in untreated versus sitagliptin-treated kidneys, respectively.
5. Analysis of variance revealed a significant ($p=0.0367$) effect of sitagliptin on renovascular responses to Ang that was strain independent.
6. Conclusion: Sitagliptin enhances renovascular responses to Ang in rats harboring SHR genes, and this effect persists in rats with diabetic nephropathy and the metabolic syndrome.

INTRODUCTION

Our previous studies indicate that Y_1 -receptor activation enhances renovascular responses to angiotensin II (Ang) in kidneys from spontaneously hypertensive rats (SHR), but not in

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kidneys from normotensive Wistar-Kyoto (WKY) rats.¹ The importance of this effect is highlighted by the finding that renal sympathetic nerve stimulation, which releases the endogenous Y₁-receptor agonist neuropeptide Y₁₋₃₆ (NPY₁₋₃₆), augments Ang-induced renovascular responses via a Y₁-receptor-mediated action in SHR, but not WKY kidneys.²

In contrast to Y₁ receptors, activation of Y₂ receptors has little effect on Ang-induced renovascular responses, even in SHR kidneys.¹ This also may be important because dipeptidyl peptidase IV (DPPIV) converts NPY₁₋₃₆ and peptide YY₁₋₃₆ (PYY₁₋₃₆), both of which are Y₁-receptor agonists, to NPY₃₋₃₆ and PYY₃₋₃₆, respectively, both of which are selective Y₂-receptor agonists.³⁻⁵ This suggests that a critical function of renal DPPIV is to regulate renovascular responses to Ang in genetically-susceptible kidneys by metabolizing endogenous Y₁-receptor agonists to Y₂-receptor agonists. In support of this concept, inhibition of DPPIV augments the ability of PYY₁₋₃₆ and NPY₁₋₃₆ to enhance renovascular responses to Ang in SHR kidneys^{6, 7}, and DPPIV inhibition can increase the renovascular response to Ang in SHR kidneys during renal sympathetic nerve stimulation.⁶

Because DPPIV inhibitors are indicated for type 2 diabetes⁸⁻¹⁰, they are used frequently in patients with the metabolic syndrome (type 2 diabetes, obesity, dyslipidemia and hypertension) and diabetic nephropathy. Therefore, it is important to determine whether in this setting DPPIV inhibition enhances renovascular responses to Ang. However, to investigate this requires an animal model with the metabolic syndrome expressed within the SHR genetic background.

The Obese-ZSF1 rat is a reliable animal model of the metabolic syndrome and diabetic nephropathy that is generated by crossing male heterozygous lean SHHF/Mcc-fa^{CP} rats with female heterozygous lean ZDF rats.¹¹ Obese offspring (Obese-ZSF1 rats) are fa/fa^{CP} at the leptin receptor gene, and lean offspring (Lean-ZSF1 rats) are either +/fa, +/fa^{CP} or +/+ at the leptin receptor gene. Accordingly, Obese-ZSF1 rats express type 2 diabetes, obesity, dyslipidemia and diabetic nephropathy, whereas Lean-ZSF1 rats do not. However, both strains inherit SHR genes from the SHHF/Mcc-fa^{CP} rat and both are hypertensive.

Because of the unique genetics of ZSF1 rats, we considered that ZSF1 rats, like SHR, may have a phenotype that includes enhancement of Ang-induced renal vasoconstriction by DPPIV inhibitors. Moreover, due to the fact that Obese-ZSF1 rats express the metabolic syndrome and diabetic nephropathy, whereas Lean-ZSF1 rats do not, Lean-ZSF1 rats provide an excellent comparator to determine whether enhancement of Ang-induced renal vasoconstriction by DPPIV inhibitors persists in the clinically-relevant setting of the metabolic syndrome and diabetic nephropathy. Accordingly, here we report on the effects of sitagliptin, a clinically important DPPIV inhibitor, on renovascular responses to Ang in Lean-ZSF1 rats versus Obese-ZSF1 rats.

METHODS

Male, 6-week-old, Obese-ZSF1 (n=10) and Lean-ZSF1 (n=11) rats were obtained from Charles River (Wilmington, MA). At 7 weeks of age rats were placed in metabolic cages, and after 24 hours of acclimatization, urine was collected for 24 hours. Next, rats were weighed, fasted overnight, and blood samples drawn from the tail vein. The following urine and blood parameters were determined: urine protein (bicinchoninic acid reagent (Pierce, Rockford, IL)); urine and plasma glucose (glucose HK assay kit (Sigma-Aldrich, St. Louis, MO)); and glycosylated hemoglobin (A1cNow+® test kit (Bayer HealthCare, Sunnyvale, CA)).

At 8 weeks of age, rats were anesthetized with Inactin, arterial blood pressure was measured (pressure transducer attached to arterial catheter) and the left kidney was isolated and

perfused with Tyrode's solution using a Hugo Sachs Elektronik-Harvard Apparatus GmbH (March-Hugstetten, Germany) kidney perfusion system as previously described.^{1, 2, 6, 7, 12} Kidneys were perfused (single pass) at a constant flow (5 ml/min), and perfusion pressure was monitored with a pressure transducer. After a 1-hour stabilization period, renovascular responses (changes in perfusion pressure) to Ang (0.1 nmol/L) were assessed by infusing Ang into the perfusate for 10 minutes. Some kidneys were pretreated for 15 minutes before administering Ang with sitagliptin (1 μ mol/L; treatment was continued until the end of the Ang infusion). Because sitagliptin is not available from chemical companies, Januvia® tablets containing sitagliptin phosphate monohydrate were purchased, pulverized and extracted with water. The extract was filtered (2 micron) and the solution tested by ion-trap mass spectrometry to confirm the presence and purity of sitagliptin.

Vasoconstrictor effects of Ang in the absence and presence of sitagliptin and in Lean-ZSF1 and Obese-ZSF1 rats were compared by an independent sampling 2-factor analysis of variance in which one factor was rat strain (2 levels, Lean-ZSF1 rats and Obese-ZSF1 rats) and the second factor was sitagliptin (2 levels, treatment or not with sitagliptin). Metabolic variables and blood pressure in Lean-ZSF1 versus Obese-ZSF1 were compared with an unpaired Student's t-test. Statistical analyses were conducted using the NCSS 2004 software (Kaysville, Utah), and the criterion for significance was $p < 0.05$. Values are means $\pm$ SEM.

RESULTS

Compared to 7-week-old Lean-ZSF1, 7-week-old Obese-ZSF1 demonstrated significant increases in body weight (262 ± 8 versus 310 ± 13 g, $p = 0.0048$), food intake (87 ± 5 versus 124 ± 3 g/kg body weight/24 hours, $p < 0.0001$), water intake (143 ± 19 versus 278 ± 37 ml/kg body weight/24 hours, $p = 0.0033$), plasma glucose (112 ± 4 versus 153 ± 9 mg/dl, $p = 0.0006$), hemoglobin A1c (4.7 ± 0.1 versus 5.8 ± 0.4 %, $p = 0.0034$), urinary glucose excretion (0.02 ± 0.003 versus 6.70 ± 1.80 g/kg body weight/24 hours, $p = 0.0016$), urine volume (36 ± 3 versus 165 ± 29 ml/kg body weight/24 hours, $p = 0.0002$) and urinary protein excretion (100 ± 7 versus 313 ± 77 mg/kg body weight/24 hours, $p = 0.0093$). Both Obese-ZSF1 and Lean-ZSF1 were hypertensive with a mean arterial blood pressure of 133 ± 7 versus 133 ± 7 mmHg, respectively ($p = 0.9535$). Thus the phenotype of the Obese-ZSF1 rats, even at 7 weeks of age was consistent with the metabolic syndrome and diabetic nephropathy.

In the isolated, perfused kidneys, baseline perfusion pressures were not significantly different among the four groups: Lean-ZSF1, 51 ± 3 mmHg ($n = 5$); sitagliptin-treated Lean-ZSF1, 51 ± 5 mmHg ($n = 6$); Obese-ZSF1, 57 ± 3 , mmHg ($n = 4$); sitagliptin-treated Obese-ZSF1, 50 ± 2 mmHg ($n = 6$). Basal perfusion pressures were lower than arterial pressure because the kidneys were perfused with Tyrode's solution which has much lower viscosity compared with blood.¹³ However, the isolated, perfused rat kidney is characterized by high vascular sensitivity to vasoconstrictors^{7, 14-16} and the vascular response of the preparation to Ang is similar to that observed in vivo.¹⁷ As shown in Figure 1, In kidneys from 8-week-old Lean-ZSF1, renovascular responses to physiological levels of Ang (0.1 nmol/L) were 3.4 ± 1.3 ($n = 5$) and 18.2 ± 5.9 ($n = 6$) mmHg in untreated versus sitagliptin-treated (1 μ mol/L) kidneys, respectively. In kidneys from 8-week-old Obese-ZSF1, renovascular responses to Ang were 5.5 ± 1.3 ($n = 4$) and 17.8 ± 8.2 ($n = 6$) mmHg in untreated versus sitagliptin-treated kidneys, respectively. 2-Factor analysis of variance (factor 1, rat strain; factor 2, sitagliptin) revealed a significant ($p = 0.0367$) effect of sitagliptin on renovascular responses to Ang that was strain independent ($p = 0.8411$, interaction term in analysis of variance). Responses to Ang, both in the presence and absence of sitagliptin, were not different between strains ($p = 0.8842$).

DISCUSSION

Worldwide, diabetes affects approximately 180 million adults, and DPPIV inhibitors afford a novel treatment for type 2 diabetes, with sitagliptin now the second leading branded oral antidiabetic agent in the United States and sales of vildagliptin in Europe rapidly increasing.¹⁸ However, DPPIV metabolizes at least 24 endogenous substrates,⁴ and the pharmacological consequences of inhibiting the metabolism of these substrates is mostly unknown but potentially important. For example, Brown et al.¹⁹ provide strong evidence that DPPIV inhibitors may increase angioedema risk in patients treated with ACE inhibitors mostly likely due to blockade of substance P metabolism.

Our previous studies suggest that DPPIV inhibitors may increase renovascular responses to Ang in genetically-susceptible kidneys by blocking the metabolism of NPY₁₋₃₆ or PYY₁₋₃₆.^{6, 7} The results of the present study confirm and extend these findings by showing that sitagliptin augments Ang-induced renal vasoconstriction in kidneys from animals with the metabolic syndrome and diabetic nephropathy, i.e., an animal model of the patient population most likely to use DPPIV inhibitors. In ZSF1 rats, this effect of sitagliptin is evident even in the absence of renal sympathetic nerve stimulation to release endogenous NPY₁₋₃₆. This is not too surprising given the fact that renal cortical tubules are a major source of renal NPY₁₋₃₆²⁰ and that the isolated, perfused rat kidney releases NPY₁₋₃₆ even under basal conditions.¹⁶ However, the source of basal endogenous NPY₁₋₃₆ and whether Ang enhances release of basal endogenous NPY₁₋₃₆ are unknown.

As described previously by us,²¹ agonists coupled to the Gi signal transduction pathway enhance renovascular responses to Ang in SHR, but not in WKY, Sprague-Dawley rats or rats with DOCA-salt hypertension. Because the Y₁ receptor mediates its effects via the Gi signal transduction pathway, most likely the observed renal effects of sitagliptin apply only to animals harboring SHR genes.

The focus of the current study was on hypertensive animals with the metabolic syndrome, and we did not examine the effects of sitagliptin in normotensive animals with the metabolic syndrome. Also, we did not investigate whether the ability of sitagliptin to enhance renovascular responses to Ang is detrimental or beneficial. However, given the well documented efficacy of inhibitors of the renin-angiotensin system to reduce the progression of diabetic nephropathy,²² it seems likely that augmenting the renovascular effects of Ang would be detrimental. Studies by Kirino et al.²³ are of great interest in this regard. These investigators show that in genetically-DPPIV-deficient rats (F344/DuCrIj rats), chronic (6 weeks) type I diabetes results in a 50% reduction in creatinine clearance compared with control rats with type I diabetes. Although the significance of our findings and the observations by Kirino et al. for diabetic patients remains to be defined, it may be that in a subpopulation of patients, sitagliptin could augment the renovascular effects of Ang and thereby reduce renal function.

Acknowledgments

Work was supported by grants from the National Institutes of Health (HL069846 and DK068575).

References

1. Dubinon JH, Mi Z, Zhu C, Gao L, Jackson EK. Pancreatic polypeptide-fold peptide receptors and angiotensin II-induced renal vasoconstriction. *Hypertension*. 2006; 47:545–551. [PubMed: 16365188]

2. Dubinon JH, Mi Z, Jackson EK. Role of renal sympathetic nerves in regulating renovascular responses to angiotensin II in spontaneously hypertensive rats. *J. Pharmacol. Exp. Ther.* 2006; 317:1330–1336. [PubMed: 16537795]
3. Berglund MM, Hipskind PA, Gehlert DR. Recent developments in our understanding of the physiological role of PP-fold peptide receptor subtypes. *Exp. Biol. Med.* 2003; 228:217–244.
4. Gorrell MD. Dipeptidyl peptidase IV and related enzymes in cell biology and liver disorders. *Clin. Sci.* 2005; 108:277–292. [PubMed: 15584901]
5. Michel MC, Beck-Sickinger A, Cox H, et al. XVI. International Union of Pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. *Pharmacol. Rev.* 1998; 50:143–150. [PubMed: 9549761]
6. Jackson EK, Mi Z. Sitagliptin augments sympathetic enhancement of the renovascular effects of angiotensin II in genetic hypertension. *Hypertension.* 2008; 51:1637–1642. [PubMed: 18443229]
7. Jackson EK, Zhang M, Liu W, Mi Z. Inhibition of renal dipeptidyl peptidase IV enhances peptide YY1–36-induced potentiation of angiotensin II-mediated renal vasoconstriction in spontaneously hypertensive rats. *J. Pharmacol. Exp. Ther.* 2007; 323:431–437. [PubMed: 17726157]
8. Augustyns K, Van der Veken P, Senten K, Haemers A. The therapeutic potential of inhibitors of dipeptidyl peptidase IV (DPP IV) and related proline-specific dipeptidyl aminopeptidases. *Curr. Med. Chem.* 2005; 12:971–978. [PubMed: 15853709]
9. Bailey CJ. Drugs on the horizon for diabetes. *Current Diabetes Reports.* 2005; 5:353–359. [PubMed: 16188170]
10. Demuth HU, McIntosh CH, Pederson RA. Type 2 diabetes--therapy with dipeptidyl peptidase IV inhibitors. *Biochim. Biophys. Acta.* 2005; 1751:33–44. [PubMed: 15978877]
11. Rafikova O, Salah EM, Tofovic SP. Renal and metabolic effects of tempol in obese ZSF1 rats--distinct role for superoxide and hydrogen peroxide in diabetic renal injury. *Metabolism.* 2008; 57:1434–1444. [PubMed: 18803950]
12. Jackson EK, Gao L, Zhu C. Mechanism of the vascular angiotensin II/ α_2 -adrenoceptor interaction. *J. Pharmacol. Exp. Ther.* 2005; 314:1109–1116. [PubMed: 15901799]
13. Bekersky I. Use of the isolated perfused kidney as a tool in drug disposition studies. *Drug Metab. Rev.* 1983; 14:931–960. [PubMed: 6360610]
14. Mi Z, Jackson EK. Effects of α - and β -adrenoceptor blockade on purine secretion induced by sympathetic nerve stimulation in the rat kidney. *J. Pharmacol. Exp. Ther.* 1999; 288:295–301. [PubMed: 9862784]
15. Gao L, Zhu C, Jackson EK. α_2 -Adrenoceptors potentiate angiotensin II- and vasopressin-induced renal vasoconstriction in spontaneously hypertensive rats. *J. Pharmacol. Exp. Ther.* 2003; 305:581–586. [PubMed: 12606648]
16. Oellerich WF, Malik KU. Neuropeptide Y modulates the vascular response to periarterial nerve stimulation primarily by a postjunctional action in the isolated perfused rat kidney. *J. Pharmacol. Exp. Ther.* 1993; 266:1321–1329. [PubMed: 8371140]
17. Jackson EK, Gillespie DG, Zhu C, Ren J, Zacharia LC, Mi Z. α_2 -Adrenoceptors enhance angiotensin II-induced renal vasoconstriction: role for NADPH oxidase and RhoA. *Hypertension.* 2008; 51:719–726. [PubMed: 18250367]
18. Grouzmann E, Livio F, Buclin T. Angiotensin-converting enzyme and dipeptidyl peptidase IV inhibitors: an increased risk of angioedema. *Hypertension.* 2009; 54:468–470. [PubMed: 19581503]
19. Brown NJ, Byiers S, Carr D, Maldonado M, Warner BA. Dipeptidyl peptidase-IV inhibitor use associated with increased risk of ACE inhibitor-associated angioedema. *Hypertension.* 2009; 54:516–523. [PubMed: 19581505]
20. Haefliger JA, Waeber B, Grouzmann E, et al. Cellular localization, expression and regulation of neuropeptide Y in kidneys of hypertensive rats. *Regul. Pept.* 1999; 82:35–43. [PubMed: 10458644]
21. Jackson EK, Herzer WA, Kost CK Jr, Vyas SJ. Enhanced interaction between renovascular α_2 -adrenoceptors and angiotensin II receptors in genetic hypertension. *Hypertension.* 2001; 38:353–360. [PubMed: 11566904]

22. Estacio RO. Renin-angiotensin-aldosterone system blockade in diabetes: role of direct renin inhibitors. *Postgrad. Med.* 2009; 121:33–44. [PubMed: 19491538]
23. Kirino Y, Sato Y, Kamimoto T, Kawazoe K, Minakuchi K, Nakahori Y. Interrelationship of dipeptidyl peptidase IV (DPP4) with the development of diabetes, dyslipidaemia and nephropathy: a streptozotocin-induced model using wild-type and DPP4-deficient rats. *J. Endocrinol.* 2009; 200:53–61. [PubMed: 18931022]

Figure 1. Renovascular responses (changes in renal perfusion pressure) to angiotensin II (Ang II; 100 pmol/L) in the absence and presence of sitagliptin (1 μ mol/L) in both Lean-ZSF1 and Obese-ZSF1 rats.