Impaired responsiveness of renal sensory nerves in streptozotocin treated rats and obese Zucker diabetic fatty rats; role of angiotensin.

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Short title: Renal sensory nerve activation impaired in diabetes

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ABSTRACT

Increasing afferent renal nerve activity decreases efferent renal nerve activity (ERNA) and increases urinary sodium excretion. Activation of renal pelvic mechanosensory nerves is impaired in streptozotocin (STZ) treated rats, model of type I diabetes. Decreased activation of renal sensory nerves would lead to increased ERNA, sodium retention and hypertension. We examined whether the reduced activation of renal sensory nerves in STZ rats was due to increased renal angiotensin activity and whether activation of the renal sensory nerves was impaired in obese Zucker diabetic fatty (ZDF) rats, model of type II diabetes. In an isolated renal pelvic wall preparation from rats treated with STZ for 2 wks, PGE$_2$ failed to increase the release of substance P, from 5±1 to 6±1 pg/min. In pelvises from Sham-STZ rats, PGE$_2$ increased substance P release from 6±1 to 13±2 pg/min. Adding losartan to the incubation bath increased PGE$_2$-mediated release of substance P in STZ rats, from 5±1 to 10±2 pg/min, but had no effect in Sham-STZ rats. In pelvises from obese ZDF rats (22-46 wks old), PGE$_2$ increased substance P release from 12.0±1.2 to 18.3±1.2 pg/min which was less than that from lean ZDF rats, 10.3±1.6 to 22.5±2.4 pg/min. Losartan had no effect on the PGE$_2$-mediated substance P release in obese or lean ZDF rats. Conclusion: The mechanisms involved in the decreased responsiveness of the renal sensory nerves in STZ rats involve activation of the renin angiotensin system in STZ but not in obese ZDF rats.

Key Words: kidney, renal sensory nerves, prostaglandin, neuropathy, substance P
INTRODUCTION

Renal mechanosensory nerves are activated by increases in renal pelvic pressure of a magnitude commonly seen during moderate volume expansion (20). The functional responses to activation of these sensory nerves include decreases in efferent renal sympathetic nerve activity (ERSNA) leading to increases in urinary sodium excretion, a renorenal reflex response (26).

Among the various mechanisms activated by increased renal pelvic pressure is induction of cyclooxygenase-2 (COX-2) leading to increased renal pelvic synthesis of PGE₂ (23,25). PGE₂ activates the cAMP - protein kinase A transduction pathway resulting in a Ca²⁺-dependent release of the neuropeptide substance P(16,22) and activation of the afferent renal nerves.

Activation of the renorenal reflex mechanism by increases in renal pelvic pressure associated with increased urine flow would facilitate the excretion of an increased sodium load by decreasing ERSNA. The importance of the renorenal reflexes in the long-term control of body fluid and sodium homeostasis was demonstrated by the salt sensitive hypertension in rats which lack intact afferent renal innervation (19). Also, suppression of afferent renal nerve activity (ARNA) which occurs in conditions of increased activation of the renin angiotensin system (15,17,18,20,21) contributes to increased ERSNA and sodium retention. Whereas, this is an appropriate response in physiological conditions of increased activation of the renin angiotensin system, including low sodium diet, suppression of these reflexes in pathological conditions of increased ANG II activity, including hypertension and congestive heart failure (17,21) would contribute to the increased ERSNA and sodium retention prevalent in these conditions.

Insulin dependent and non-insulin dependent diabetes mellitus are referred to as Type I and Type II diabetes mellitus, respectively. Rats made hyperglycemic by streptozotocin (STZ)
treatment are an insulin deficient model of type I diabetes. The euglycemic obese Zucker rat is the parent strain from which the obese Zucker diabetic fatty (ZDF) rat is developed. The ZDF rat is an often used animal model for human Type II diabetes. Type II diabetes is the most common diabetes in USA and is characterized by obesity, insulin resistance and eventual failure of pancreatic islet β-cells to produce enough insulin to meet demand resulting in hyperglycemia (12). The obese ZDF rat is hyperglycemic and initially hyperinsulinemic (40,41,46). Eventually the insulin levels decline due to pancreas/β cell exhaustion. The obese Zucker rat is insulin resistant and dyslipidaemic but not hyperglycemic. Diabetes, type I and II, is associated with markedly increased incidence of cardiovascular diseases, including hypertension (3,8,11,55). There is the considerable evidence for increased angiotensin II activity in type I and type II diabetes and inhibitors of the renin angiotensin system are commonly used in diabetic patients to treat hypertension and other cardiovascular diseases, including diabetic nephropathy (1,2,4,11,28,32,33).

Diabetic neuropathy is a serious and common complication in both type I and type II diabetes, and encompasses multiple organs (57). It is multifactorial and has been attributed to vasculature diseases leading to nerve ischemia and/or a combination of metabolic defects associated with increased influx of glucose through the aldose reductase pathway (49). There is considerable evidence for impaired sensitivity of carotid and aortic baroreceptor reflexes and cardiac chemoreceptor reflexes in type I diabetes mellitus in both patients and STZ-treated rats (11,39,43,56). Likewise, impaired baroreflexes leading to increased ERSNA and hypertension have been demonstrated in obese ZDF and Zucker rats and patients with type II diabetes (42,44,45).
There is a decreased responsiveness of renal mechanosensory nerves during volume expansion in STZ rats (9). The impaired renorenal reflexes in these rats may contribute to the altered homeostatic regulation of arterial pressure and sodium balance in STZ rats. Therefore, the current study was designed to examine the mechanisms involved in the decreased sensitivity of the renal mechanosensory nerves in STZ rats. In view of the increased activity of the renin angiotensin system in kidneys of STZ rats (2, 13) together with our studies showing marked inhibitory effects of ANG II on the activation of renal mechanosensory nerves (20), we hypothesized that increased ANG II activity contributes to the decreased responsiveness of renal mechanosensory nerves in STZ rats. Because early onset of diabetes in STZ rats is characterized by increased arterial pressure, renal blood flow and natriuresis (6), we used an isolated renal pelvic wall preparation to minimize the influence of the cardio-renal circulation on the activation of the renal sensory nerves. By examining the release of substance P produced by PGE_2, this preparation allows us to determine whether the reduced responsiveness of the renal mechanosensory nerves observed \textit{in vivo} is related to mechanisms distal to renal pelvic PGE_2 synthesis.

In view of the salt sensitive hypertension in obese ZDF and Zucker rats (8,34) together with decreased sensitivity of the carotid baroreceptor reflexes (44,45) and increased activity of the renin angiotensin system (5,33,47), we also examined whether the responsiveness of the renal sensory nerves is altered in these rats.

METHODS
Male Sprague Dawley rats were obtained from Harlan (Indianapolis, IN) and obese and lean Zucker rats, and ZDF rats from Charles River Laboratories (Wilmington, MA). Rats were provided food (all rats, except obese ZFD rats: Harlan Teklad no. 7001, obese ZDF rats: Harlan Teklad 7013) and tap water ad libitum. The diet fed to the ZDF obese rats has a slightly higher fat content (6% vs. 4%) which is required for the development of diabetes in this model.

The experimental protocols were approved by the Institutional Animal Care and Use Committee and performed according to the “Guide for the Care and Use of Laboratory Animals” from the National Institutes of Health.

*Substance P release from an isolated renal pelvic wall preparation.*

The procedures for measuring the release of substance P from an isolated rat renal pelvic wall preparation have been previously described in detail (15-18,20,22,24,25). In brief, the rats were anesthetized with pentobarbital sodium (0.2 mmol/kg i.p. Abbott Laboratories). The renal pelvises dissected from the kidneys were placed in wells containing 400 µl HEPES buffer (25 mM HEPES, 135 mM NaCl, 3.5 mM KCl, 2.5 mM CaCl₂, 1 mM MgCl₂, 3.3 mM d-glucose, 0.1 mM ascorbic acid, 0.1% BSA, 10 µM dl-thiorphan, 1 mM Phe-Ala, 50 µM p-chloromercuriphenylsulfonic acid, pH 7.4) maintained at 37°C. Indomethacin, 0.14 mM, was present in the incubation bath to minimize the influence of endogenous PGE₂ on substance P release. Each well contained the pelvic wall from one kidney.

The renal pelvic walls were allowed to equilibrate for 130 minutes. The incubation medium was replaced with fresh HEPES every 10 minutes for the first 120 min and every 5 min thereafter. The incubation medium was collected in siliconized vials and stored at -80°C for later
analysis of substance P. The experimental protocol consisted of four 5-min control periods, one 5-
min experimental period and four 5-min recovery periods. PGE\textsubscript{2} was added to the incubation bath
to both the ipsilateral and contralateral pelvises during the experimental periods.

\textit{Induction of Experimental Diabetes}

Sprague Dawley rats were randomized to receive either streptozotocin (STZ), single i.p.
injection, 55 mg/kg in 0.9\% saline, adjusted to pH 4.0 with 0.2 M sodium citrate, or vehicle
(Sham-STZ) under light halothane anesthesia. The acute experimental studies, detailed below,
were performed two weeks later. The studies were divided into two main groups. In the first
group, we examined whether systemic treatment with an AT1 receptor (AT1-R) antagonist for
two weeks modulated the PGE\textsubscript{2}-mediated release of substance P from the renal pelvic wall.
Because the results from these studies suggested that long-term systemic administration of an
AT1-R antagonist enhanced the PGE\textsubscript{2}-mediated release of substance P in STZ rats, we examined
whether acute administration of an AT1-R antagonist to the incubation bath modulated the PGE\textsubscript{2}-
mediated release of substance P in STZ and Sham-STZ rats.

\textit{Experimental Protocols}

\textit{Experiment I: Effects of long-term systemic administration of an AT1-R antagonist on the
PGE\textsubscript{2} mediated substance P release in STZ and Sham-STZ rats.} Four groups of litter mates were
studied. In the first two groups consisting of STZ rats (n=9) and Sham-STZ rats (n=6) the AT1-R
antagonist candesartan, 100 mg/kg diet, was added to the diet for a period of two weeks. The next
two groups consisting of STZ rats (n=6) and Sham-STZ rats (n=6) were untreated and served as
controls. On the day of the acute experiment (see above), rats were anesthetized and catheters
placed in the femoral artery and vein for measurements of arterial pressure and administration of
ANG II to test the efficacy of the candesartan treatment to block AT1-R. To minimize the influence of acute administration of ANG on the PGE₂-mediated release of substance P (20) in the subsequent in vitro studies, ANG II was injected into the femoral vein immediately after the renal artery and vein were ligated and kidneys removed.

Ipsilateral and contralateral renal pelvises from all groups were incubated in HEPES/indomethacin buffer as described above. During the experimental period, the ipsilateral and contralateral pelvises were exposed to PGE₂, 0.14 µM (20).

**Experiment II: Effects of acute renal pelvic administration of an AT1-R antagonist on the PGE₂ mediated substance P release in STZ and Sham-STZ rats.** Three groups of litter mates were studied. The first group consisted of STZ rats (n=10), the second group STZ rats (n=12) treated with insulin twice a day for two weeks, 2 units at 8:00AM and 4 units at 4:30PM, and the third group Sham-STZ rats (n=8). The ipsilateral renal pelvis was incubated in HEPES/indomethacin buffer as described above. The contralateral renal pelvis was incubated in HEPES/indomethacin buffer containing the AT1-R antagonist losartan, 0.44mM, throughout the control, experimental and recovery periods. During the experimental period, PGE₂ at 0.14 µM was administered to both pelvises in all three groups.

**Experiment III: Effects of acute renal pelvic administration of an AT1-R antagonist on the PGE₂ mediated substance P release in obese and lean ZDF rats.** Four groups were studied. In the first two groups, ipsilateral and contralateral renal pelvises from obese ZDF (n=9) and lean ZDF (n=7) rats were incubated in HEPES/indomethacin buffer as described above. In the next two groups, the ipsilateral pelvises from obese ZDF (n=12) and lean ZDF (n=8) rats were incubated in HEPES/indomethacin buffer as described above. The contralateral renal pelvis was incubated in
HEPES/indomethacin buffer containing losartan, 0.44 mM, throughout the control, experimental and recovery periods. During the experimental period, PGE$_2$ at 0.14 µM was administered to both pelvises in all four groups.

**Experiment IV: Effects of acute renal pelvic administration of an AT1-R antagonist on the PGE$_2$ mediated substance P release in obese and lean Zucker rats.** Two groups were studied. The ipsilateral pelvises from obese Zucker (n=16) and lean Zucker (n=12) rats were incubated in HEPES/indomethacin buffer as described above. The contralateral renal pelvises of 8 of 16 obese Zucker rats and 9 of 11 lean Zucker rats were incubated in HEPES/indomethacin buffer containing losartan, 0.44mM, and the remaining contralateral pelvises in HEPES/indomethacin buffer throughout the control, experimental and recovery periods. During the experimental period, PGE$_2$ at 0.14 µM was administered to both pelvises in the two groups.

At the end of each experiment in Experiments I, III and IV, the responsiveness of the renal sensory nerves to capsaicin, 8.5 µM, an activator on non-selective cation channels on sensory nerve fibers, was examined to test the specificity of the diabetes-induced modulation of the PGE$_2$-mediated release of substance P.

**Drugs.**

Losartan was supplied by Merck & Co., Inc (Rathway, NJ) and candesartan by AstraZeneca (Wilmington, DE). Substance P antibody (IHC 7451) was acquired from Penninsula Laboratories (San Carlos, CA). All other agents were from Sigma Chemicals (St. Louis, MO) unless otherwise stated. Indomethacin was dissolved together with Na$_2$CO$_3$ (2:1 weight ratio) in HEPES buffer and all other agents in incubation buffer.
Analytical Procedures

On the day of the acute experiment, nonfast blood glucose levels were determined in the anesthetized rat by the use of glucose oxidase reagent strips (Lifescan, Milpitas, CA or Accu-Chek, Roche, Indianapolis, IN). Substance P in the incubation medium was measured by ELISA, as previously described in detail (15-18,20,22,24).

Statistical Analysis

The release of substance P during the experimental period was compared with the substance P release during the control and recovery periods using Friedman 2-way analysis of variance and shortcut analysis of variance. In the experiments where both ipsilateral and contralateral pelvises were treated with HEPES/indomethacin buffer, the release of substance P from the ipsilateral and contralateral kidneys was averaged, thus n=number of rats. The Wilcoxon matched-pairs signed-rank test was used to compare the PGE2 -induced increases in renal pelvic release of substance P between the ipsilateral pelvis treated with buffer and contralateral pelvises treated with losartan. Mann Whitney U-test was used to compare the differences in substance P release between groups. A significance level of 5% was chosen. Data in text and figures are expressed as means ± SE (48,52).

RESULTS

Experimentally induced diabetes

As shown in Table 1, blood glucose was higher and body weight lower in STZ rats and in STZ rats treated with candesartan compared to their Sham-STZ litter mates. Body weight in STZ rats treated with insulin and Sham-STZ rats was similar. The lower blood glucose value in STZ rats
treated with insulin reflects the fact that blood glucose was measured less than 60 minutes after insulin injection. Baseline substance P release was similar in all groups.

**Experiment I: Effects of long-term systemic administration of an AT1-R antagonist on the PGE₂ mediated substance P release in STZ and Sham-STZ rats.** Previous studies have shown a decreased ARNA response to increases in renal pelvic pressure in STZ rats (9). We hypothesized that this impairment was due to mechanisms at the peripheral sensory nerve endings involving increased ANG II activity. We tested this hypothesis by comparing the PGE₂-mediated release of substance P from renal pelvises derived from candesartan-treated rats with that from pelvises derived from vehicle-treated rats. As shown in Fig. 1, PGE₂ resulted in a reversible release of substance P in vehicle-treated Sham-STZ rats that was significantly greater than that produced in vehicle-treated STZ rats (P<0.01). Adding candesartan to the diet for 2 weeks enhanced the PGE₂-mediated increase in substance P release in STZ rats (P<0.01) but had no effect in Sham-STZ rats. The increase in PGE₂-mediated release of substance P in candesartan treated STZ rats was slightly less than that produced by PGE₂ in candesartan treated Sham-STZ (P<0.05). As shown in table 2, the increase in substance P release produced by capsaicin was also reduced in vehicle-treated STZ rats (P<0.05 vs., sham-STZ), as tested at the end of the experiment. There was no difference in the substance P response to capsaicin in the candesartan treated STZ-rats compared to that in vehicle-treated and candesartan-treated Sham-STZ rats.

ANG II, 100 ng/kg i.v., increased mean arterial pressure from 108±10 to 143±8 mmHg and from 105±9 to 139±10 mmHg in untreated Sham-STZ and STZ rats, respectively. In the candesartan-treated Sham-STZ and STZ rats, ANG II had no effect on mean arterial pressure, from 94±5 to 94±7 mmHg and from 106±4 to 103±5 mmHg, respectively.
Experiment II: Effects of acute renal pelvic administration of an AT1-R antagonist on the PGE$_2$ mediated substance P release in STZ and Sham-STZ rats. Because the studies in Experiment I showed that long-term oral administration of candesartan prevented, at least in part, the impairment of the PGE$_2$-mediated release of substance P in STZ rats, we then examined whether acute administration of an AT1-R antagonist would modify the responsiveness of the renal sensory nerves in STZ rats. Furthermore we examined whether the impaired activation of the renal sensory nerves was due to STZ, per se, or to the induced hyperglycemia by treating STZ rats with insulin twice a day during the two-week period. Insulin prevented the hyperglycemia produced by STZ injection (Table 1). As shown in Fig. 2 in vehicle-treated STZ rats, PGE$_2$ resulted in an increase in substance P that was reduced to a similar extent as in vehicle-treated STZ rats in Experiment I. In STZ rats treated with insulin for two weeks to prevent hyperglycemia (Table 1), the PGE$_2$-mediated release of substance P was similar to that in Sham-STZ rats (Fig. 2, Table 3). Adding losartan to the incubation bath had no effect on the PGE$_2$-induced release of substance P in insulin-treated STZ rats or Sham-STZ rats but markedly enhanced the PGE$_2$-induced substance P release in STZ rats, albeit the increase in substance P release being less than that produced in insulin-treated STZ rats (P<0.01).

Experiment III: Effects of acute renal pelvic administration of an AT1-R antagonist on the PGE$_2$ mediated substance P release in obese and lean ZDF rats. The obese ZDF rat being an animal model of type II diabetes is associated with widespread neuropathy (49,57). We therefore examined whether the responsiveness of the renal pelvic sensory nerves is impaired in these rats. An impairment of the renal sensory nerves could contribute to altered water and sodium homeostasis and thereby the hypertension commonly seen in these rats and humans with type II
Because inhibition of the renin angiotensin system improved thermal nociception and increased sensory nerve conduction in obese ZDF rats, we examined whether an AT1-R antagonist would modify the responsiveness of the renal sensory nerves.

As shown in Table 1, the obese ZDF rats were hyperglycemic. The age of the lean and obese ZDF rats ranged from 22 to 46 weeks. There was no correlation between baseline substance P release, the magnitude of the PGE2-mediated release of substance P and age in lean or obese ZDF rats. PGE2 resulted in a reversible increase in substance P release from renal pelvises derived from either obese or lean ZDF rats (Fig. 3). However, the PGE2-mediated release of substance P from pelvises derived from obese ZDF rats was significantly less than that from pelvises from lean ZDF rats. A similar reduced increase in PGE2-mediated release of substance P was observed in an additional group of obese ZDF rats (Fig. 4). Adding losartan to the bath of the contralateral pelvis from these obese ZDF rats had no effect on the PGE2-mediated substance P release. Likewise, the PGE2-mediated release of substance P from pelvises derived from lean ZDF rats was similar in the absence and presence of losartan in the bath (Fig. 4).

Experiment IV: Effects of acute renal pelvic administration of an AT1-R antagonist on the PGE2 mediated substance P release in obese and lean Zucker rats. The obese Zucker rat is the parent strain from which the obese ZDF rat was derived. Similar to the ZDF rats, baseline substance P release and the PGE2-mediated release of substance P were independent of the age of rats. In contrast to the obese ZDF rat, the obese Zucker rat is not hyperglycemic (Table 1). In contrast to the findings in obese ZDF rats, PGE2 produced a similar increase in substance P release from pelvises derived from obese and lean Zucker rats (Fig. 5). Losartan had no effect on the PGE2-mediated substance P release in obese or lean Zucker rats (Table 4).
Baseline substance P release was similar in ZDF and Zucker rats and there were no differences between the increases in substance P produced by capsaicin in obese and lean ZDF and Zucker rats (Table 2).

DISCUSSION

The present study shows that PGE\(_2\) added to the bath of isolated renal pelvises resulted in a reduced release of substance P from pelvises derived from STZ rats and obese ZDF rats, which represent animal models of type I and type II diabetes mellitus, respectively. Long-term oral administration or acute local administration of an AT1-R antagonist partially restored the PGE\(_2\)-mediated release of substance P in STZ rats. However, the AT1-R antagonist had no effect on the PGE\(_2\)-mediated release of substance P from pelvises derived from obese ZDF rats. These studies suggest that the responsiveness of the renal sensory nerves is reduced in hyperglycemic rats by a mechanism distal to PGE\(_2\) synthesis at the peripheral renal sensory nerve endings. In early stage of type I diabetes (STZ rats), the decreased responsiveness of the renal pelvic sensory nerves is due to a mechanism(s) involving, at least in part, increased ANG II-mediated activation of AT1-R. In obese ZDF rats, the impaired responsiveness of the renal sensory nerves in obese ZDF rats does not involve increased activation of the renin angiotensin system.

In the kidney, the majority of the sensory nerves containing substance P and calcitonin gene related peptide are located in the renal pelvic wall (16,24,29). We have consistently shown that the mechanisms activating renal pelvic mechanosensory nerves \textit{in vivo} can be accurately replicated in studies using the isolated renal pelvic wall preparation (16-18,20,22,24). The advantage of the isolated renal pelvic wall preparation relates to the fact that it minimizes the
influences from the systemic and renal circulation and the central nervous system. Early onset diabetes in the STZ rat is characterized by increased renal blood flow, glomerular filtration rate and polyuria (6), all factors that could alter the responsiveness of the renal mechanosensory nerves.

In agreement with the *in vivo* studies (9), the current *in vitro* studies showed decreased responsiveness of the renal sensory nerves in the hyperglycemic but not euglycemic STZ rat. Our previous studies in euglycemic normal rats showing that a urinary mannitol concentration of 1800 mM has no effect on the activity of renal pelvic mechanosensory nerves (27) suggest that the impaired responsiveness of renal mechanosensory nerves (9) is not related to high urinary glucose concentration. In STZ rats, the natriuretic response to acute volume expansion is impaired, at least in part, due to reduced fall in ERSNA (39). The observation that an increase in renal interstitial pressure of the same magnitude as that produced by volume expansion also results in reduced excretory responses in STZ vs. Sham-STZ rats (38), suggests that the decreased responsiveness of the renal mechanosensory nerves contributes to the impaired excretory response to acute volume expansion in these rats.

Studies in type I diabetic patients suggest an important role for hyperglycemia stimulating the renin angiotensin system (32). The increased arterial pressure and renal vascular resistance in these patients are reduced by losartan. Also, there is evidence for a role of increased ANG II activity in STZ-induced neuropathy. Systemic administration of ATR-1 antagonists or converting enzyme inhibitors improved motor and sensory nerve conduction velocity possibly by increasing neural blood flow (10,31). The current studies, performed in rats with early stage type I diabetes, showed that two-weeks’ systemic administration or acute local administration of two different
AT1-R antagonists produced similar enhancements of the PGE$_2$-mediated release of substance P. Our data further suggest that the enhancement produced by the AT1-R antagonists of the PGE$_2$-induced release of substance P involves a mechanism(s) located distal to PGE$_2$ synthesis in the renal pelvic wall. This hypothesis is supported by our previous studies showing that ANG II impairs PGE$_2$-mediated activation of adenylyl cyclase via a pertussis toxin sensitive effect (18). Similar to the impaired PGE$_2$-mediated substance P release in STZ rats, the release of substance P produced by capsaicin was suppressed in STZ rats and improved by the AT1-R antagonist candesartan. These data suggest that the inhibitory effects of the renin angiotensin system on the responsiveness of renal sensory nerves are not limited to PGE$_2$-mediated activation of EP4 receptors (16) but also involve activation of transient receptor potential vanilloid receptors (TRPV1) in the renal pelvic wall.

Although, the reduced responsiveness of the renal sensory nerves to PGE$_2$ was improved, it was not completely normalized by systemic or local pelvic administration of an AT1-R antagonist in STZ rats. We can not exclude the possibility that STZ-induced increase in collagenous tissue in the renal pelvic wall (9), may have impaired the PGE$_2$-induced activation of EP4 receptors. Although, the similar baseline renal pelvic release of substance P in Sham-STZ and STZ rats may argue against the idea that the decreased responsiveness of the renal sensory nerves in STZ rats is due to reduced substance P content, there is evidence in other tissues, e.g. trigeminal ganglion and retina, for substance P content being reduced in STZ rats even before morphological changes are present in peripheral nerves (53,54). The decrease in substance P content in the sciatic nerve in the absence of changes in the expression of lumbar dorsal root ganglia in STZ rats would suggest that the changes in the neuropeptide content are post-
transcriptional (7,53,54). Taken together, we hypothesize that the mechanisms involved in the decreased responsiveness of renal sensory nerves in STZ-induced diabetes are multifactorial and involve activation of the renin angiotensin system and possibly also reduced substance P content, the increased activity of the renin angiotensin system being the dominant mechanism in early stage diabetes type I.

Baseline renal pelvic substance P release did not differ within each group, i.e., Sham-STZ and STZ rats, lean/obese ZDF rats and lean/obese Zucker rats, but there was a difference in baseline renal pelvic substance P release among the groups, baseline substance P release being slightly lower in the Sham-STZ and STZ rats vs., the lean/obese ZDF and Zucker rats. However, it is unlikely that the different responses to PGE2 and losartan observed in the STZ rats and obese ZDF and Zucker rats are related to baseline renal pelvic substance P release or pelvic substance P content. This notion is supported by the similar increases in substance P release produced by PGE2 in the Sham-STZ rats, lean ZDF rats and lean Zucker rats despite baseline substance P release being lower in the Sham-STZ rats.

The obese ZDF rat is hyperglycemic from 8 weeks’ of age. Initially, they are hyperinsulinemic but by >22 wks of age, their insulin levels are below those of aged-matched lean ZDF rats. They are also hyperlipidemic. The ZDF rats are genetically identical to obese Zucker rats, but the latter are not hyperglycemic (40,41,46). In view of the salt sensitive hypertension and impaired sensitivity of the baroreceptor reflexes in obese ZDF and Zucker rats (8,44,45), we speculated that the responsiveness of the renal sensory nerves may be decreased. Our hypothesis was confirmed but only in the obese ZDF rats. In these rats, the PGE2-mediated release of substance P was significantly less than that in age-matched lean ZDF rats. However, the
difference between the PGE\textsubscript{2}-induced increase in substance P release between the obese and lean ZDF rats was much less than that between STZ and Sham-STZ rats. In obese Zucker rats, the slightly smaller increase in PGE\textsubscript{2}–mediated release of substance P compared to that in lean Zucker rats did not reach statistical significance. These data suggest that the decreased responsiveness of the renal sensory nerves in obese ZDF rats is associated with hyperglycemia.

Diabetic neuropathy is common in type II diabetes (49) and is an important contributor to the impairment of the baroreceptor reflexes in patients with type II diabetes (42). The peripheral nerve dysfunction in obese ZDF rats involves reduced endoneurial blood flow (36,37). Long-term treatment with angiotensin converting enzyme inhibitors improves both motor and sensory nerve conduction velocity and endoneurial blood flow in obese ZDF rats (37). There is evidence for enhanced depressor and natriuretic responses produced by AT1-R antagonists with mild hyperglycemia (51). However, whether intrarenal ANG II levels are increased in rats with type II diabetes is controversial. Both increased (47) and unchanged (50) intrarenal ANG II levels have been reported in obese Zucker and ZDF rats, respectively. The current studies failed to show a beneficial effect of an acute blockade of the AT1-R receptors on the responsiveness of the renal pelvic sensory nerves in obese ZDF rats. The increase in PGE\textsubscript{2}-mediated release of substance P release was similar in the absence and presence of losartan in the incubation bath containing the pelvises derived from obese ZDF rats. The similar increases in substance P release produced by capsaicin in obese and lean ZDF and Zucker rats may suggest that neuropathy may not be the main cause for the reduced responsiveness of the renal sensory nerves in obese ZDF rats.

Although not examined in the present study, there is considerable evidence for diabetic nephropathy in obese ZDF rats (5,14,58). Progressive renal injury starts at 18-20 weeks of age
and significant kidney damage is observed at 30-35 weeks of age, the average age of the ZDF rats in the current study. The majority of studies examining the effects of long-term treatment with inhibitors of the renin angiotensin system show that the diabetic-induced nephropathy is significantly reduced by inhibition of the renin angiotensin system in animals and patients with type II diabetes (4, 5, 28, 33, 35). In a limited study of 4 obese ZDF rats, we examined the effects of 12 weeks’ treatment with the converting enzyme inhibitor enalapril, 400 mg/kg/diet, on the PGE₂-mediated increase in substance P release. In this limited number of rats, PGE₂ increased the release of substance P from 4.2±1.2 to 10.1±2.2 pg/min. In 4 age-matched untreated ZDF rats, the increase in substance P release was less, from 10.3±2.7 to 12.0±3.6 pg/min. Although the marked difference in baseline substance P release between these two groups of rats, makes a comparison between the two groups difficult, it is unlikely that the suppressed increase in PGE₂-mediated release of substance P in untreated ZDF rats is due to the higher baseline substance P release, per se. Studies in non-diabetic rats, including the current lean ZDF and Zucker rats, show marked increases in PGE₂-mediated substance P release from a baseline similar to that in the untreated obese ZDF rats. In view of the result from this limited study and the considerable evidence for significant nephropathy (5, 14, 58), including hydronephrosis (30) in obese ZDF rats, we speculate that the reduced responsiveness of the renal sensory nerves in these rats is, at least in part, related to diabetes-induced nephropathy.

**Perspectives**

Increases in ERSNA increase ARNA (24). The increased ARNA exerts a negative feedback control of ERSNA via activation of the renorenal reflexes (26) in the overall goal of maintaining a low level of ERSNA, so as to limit sodium retention. In early onset type I diabetes,
there is an increased activation of the renin angiotensin system (6,13). The ANG II-induced impairment of the natriuretic renorenal reflexes may be an appropriate response in early diabetes in STZ rats to offset the increased renal blood flow, glomerular filtration rate and urinary sodium excretion commonly seen in early diabetes in STZ rats. However, an impairment of the renorenal reflexes in later stage of diabetes in STZ rats and in obese ZDF rats would contribute to increased sympathetic nerve activity and hypertension prevalent in diabetes. Long-term treatment with an inhibitor of the renin angiotensin system prevented the reduction in the responsiveness of the renal sensory nerves in obese ZDF rats. Thus, our data suggest that the well-known beneficial effects of these drugs on diabetic nephropathy include preservation of the responsiveness of the renal sensory nerves, activation of which will contribute to the renal control of body fluid and sodium homeostasis via the natriuretic renorenal reflexes.
ACKNOWLEDGMENT

This work was supported by grants from the Department of Veterans Affairs to Drs. Kopp and Yorek and The National Institutes of Health, Heart, Lung and Blood Institute, RO1 HL66068 and Diabetes and Digestive and Kidney Diseases, DK073990. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung and Blood Institute or the National Institutes of Health.
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FIGURE LEGENDS

Figure 1. Effects of candesartan, 400 mg/kg diet for two weeks, (dotted lines) on the PGE₂-mediated release of substance P from renal pelvises derived from rats treated with streptozotocin (STZ) for two weeks (left panel) or vehicle (Sham-STZ, left panel). SP, substance P; CNT, control; REC, recovery, each the average of four 5-min periods; **P<0.01 vs., CNT and REC, ‡P< 0.01, the PGE₂-mediated increase in substance P release from pelvises derived from candesartan-treated vs. vehicle-treated STZ rats.

Figure 2. Effects of PGE₂, 0.14 µM, on substance P release from renal pelvises derived from STZ rats (left panel) and STZ rats treated with insulin for two weeks (right panel) in the absence (solid lines) and presence (dotted lines) of losartan, 0.44 mM in the incubation bath. **P<0.01 vs., CNT and REC, †P< 0.05, the PGE₂-mediated increase in substance P release from losartan-treated pelvises vs. vehicle-treated pelvises derived from STZ rats.

Figure 3. Effects of PGE₂, 0.14 µM, on substance P release derived from pelvises from obese (dotted lines) and lean (solid lines) ZDF rats. **P<0.01 vs., CNT and REC; †P< 0.05, the PGE₂-mediated increase in substance P release from pelvises derived from obese vs., lean ZDF rats.

Figure 4. Effects of PGE₂, 0.14 µM, on substance P release from renal pelvises derived from obese (left panel) and lean (right panel) ZDF rats in the absence (solid lines) and presence (dotted
lines) of losartan, 0.44 mM in the incubation bath. **P<0.01 vs., average of CNT and REC.

Figure 5. Effects of PGE₂, 0.14 μM, on substance P release derived from pelvises from obese (dotted lines) and lean (solid lines) Zucker rats. **P<0.01 vs., CNT and REC.
Table 1: Characteristics of STZ, Sham-STZ, ZDF obese and lean, and Zucker obese and lean rats.

<table>
<thead>
<tr>
<th></th>
<th>Blood glucose mg/dl</th>
<th>Body Weight, g</th>
<th>age, days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STZ vehicle, n=6</td>
<td>&gt;400#*</td>
<td>244±7*</td>
<td>65±1</td>
</tr>
<tr>
<td>Sham-STZ vehicle, n=6</td>
<td>135±7</td>
<td>281±10</td>
<td>58±3</td>
</tr>
<tr>
<td>STZ candesartan, n=9</td>
<td>&gt;400#**</td>
<td>251±9*</td>
<td>66±1</td>
</tr>
<tr>
<td>Sham-STZ candesartan, n=6</td>
<td>135±7</td>
<td>285±8</td>
<td>63±2</td>
</tr>
<tr>
<td><strong>Experiment II</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STZ, n=10</td>
<td>364±16**</td>
<td>271±7**</td>
<td>93±1</td>
</tr>
<tr>
<td>STZ+insulin, n=12</td>
<td>40±5</td>
<td>313±7</td>
<td>92±1</td>
</tr>
<tr>
<td>Sham-STZ, n=8</td>
<td>103±6</td>
<td>314±6</td>
<td>93±1</td>
</tr>
<tr>
<td><strong>Experiment III</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZDF-obese, n=21</td>
<td>369±16**</td>
<td>376±8**</td>
<td>237±11</td>
</tr>
<tr>
<td>ZDF-lean, n=15</td>
<td>92±5</td>
<td>477±14</td>
<td>253±14</td>
</tr>
<tr>
<td><strong>Experiment IV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zucker-obese, n=16</td>
<td>104±7</td>
<td>677±67*</td>
<td>242±18</td>
</tr>
<tr>
<td>Zucker-lean, n=12</td>
<td>96±6</td>
<td>415±37</td>
<td>265±21</td>
</tr>
</tbody>
</table>

# Accu-Chek, Roche, Indianapolis, IN used to determine blood glucose in these rats resulted in values >400 mg/dl in all diabetic rats in this group. Lifescan, Milpitas, CA was used in the remaining rats. *P<0.05, STZ vehicle vs. Sham-STZ vehicle; STZ candesartan vs. Sham-STZ candesartan; Zucker-obese vs. Zucker-lean, ** P<0.01, STZ candesartan vs. Sham-STZ candesartan; STZ vs. STZ+insulin; STZ vs. Sham-STZ; ZDF-obese vs. ZDF-lean.
Table 2: Substance P release from an isolated renal pelvic wall preparation in the absence (control) and presence of capsaicin, 8.5 μM, in the incubation bath.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Control</th>
<th>Capsaicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STZ vehicle, n=6</td>
<td>3.8±0.3</td>
<td>6.4±0.5*†</td>
</tr>
<tr>
<td>Sham-STZ vehicle, n=6</td>
<td>5.8±1.3</td>
<td>14.8±2.4*</td>
</tr>
<tr>
<td>STZ candesartan, n=9</td>
<td>5.0±0.9</td>
<td>11.1±1.9**</td>
</tr>
<tr>
<td>Sham-STZ candesartan, n=6</td>
<td>4.6±0.7</td>
<td>13.1±1.9*</td>
</tr>
<tr>
<td>Experiment III</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZDF-obese, n=21</td>
<td>9.1±1.0</td>
<td>19.7±2.1**</td>
</tr>
<tr>
<td>ZDF-lean, n=15</td>
<td>9.3±1.3</td>
<td>20.9±2.8**</td>
</tr>
<tr>
<td>Experiment IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zucker-obese, n=14</td>
<td>8.8±1.3</td>
<td>20.8±3.4**</td>
</tr>
<tr>
<td>Zucker-lean, n=11</td>
<td>8.3±1.8</td>
<td>19.7±3.0**</td>
</tr>
</tbody>
</table>

Substance P release, pg/min, * P<0.05, ** P<0.01, capsaicin vs., control; † P<0.05, increase in substance P release produced by capsaicin in STZ vehicle vs., Sham-STZ vehicle
Table 3. Effects of PGE$_2$, 0.14 μM on renal pelvic release of substance P in the presence of vehicle and losartan in the incubation bath containing the ipsilateral and contralateral pelvises, respectively, of Sham-STZ rats.

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>losartan, 0.44mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNT</td>
<td>PGE$_2$</td>
</tr>
</tbody>
</table>
| Substance P release, pg/min; CNT, control and REC, recovery, each average of four 5-min periods | 7.3±1.9 | 14.4±3.2** | 7.2±2.3 | 5.2±1.1 | 12.1±2.4** | 5.4±1.0 | ** P<0.01, PGE$_2$ vs. control and recovery, n=8.
Table 4. Effects of PGE$_2$, 0.14 µM on renal pelvic release of substance P in the presence of vehicle and losartan in the incubation bath containing the ipsilateral and contralateral pelvises, respectively, of obese and lean Zucker rats.

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>losartan, 0.44mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNT</td>
<td>PGE$_2$</td>
</tr>
<tr>
<td>obese Zucker, n=8</td>
<td>8.7±1.6</td>
<td>15.9±3.5**</td>
</tr>
<tr>
<td>lean Zucker, n=9</td>
<td>11.5±1.5</td>
<td>18.4±2.4**</td>
</tr>
</tbody>
</table>

Substance P release, pg/min; CNT, control and REC, recovery, each average of four 5-min periods ; *P<0.05, ** P<0.01, PGE$_2$ vs. control and recovery.
Effects of oral candesartan on PGE$_2$-mediated substance P release in streptozotocin (STZ) rats and Sham-STZ rats

Figure 1
Effects of renal pelvic administration of losartan on PGE$_2$-mediated substance P release in STZ rats and insulin treated STZ rats

**Figure 2**
Figure 3

PGE2-mediately substance P release in obese and lean Zucker Diabetic Fatty (ZDF) rats

- lean ZDF rats, n=7
- obese ZDF rats, n=9
Effects of renal pelvic administration of losartan on PGE$_2$-mediated substance P release in obese and lean Zucker diabetic fatty (ZDF) rats

![Graph showing effects of losartan on SP release in obese and lean ZDF rats.](figure4)

- **Obese ZDF**: Graph showing SP release in obese Zucker diabetic fatty (ZDF) rats with vehicle and losartan treatments. The y-axis represents SP release in pg/min, and the x-axis represents time points (CNT, PGE2, REC). The graph indicates a significant decrease in SP release with losartan compared to vehicle. The sample size (n=12) is noted.

- **Lean ZDF**: Graph showing SP release in lean Zucker diabetic fatty (ZDF) rats with vehicle and losartan treatments. Similar to the obese group, the graph shows a significant decrease in SP release with losartan. The sample size (n=8) is noted.

*Significance indicated by ** for p < 0.01.*
Figure 5

PGE$_2$ - mediated substance P release in obese and lean Zucker rats

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- lean Zucker rats, n=12
- obese Zucker rats, n=16

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