Early potential impairment of renal sensory nerves in streptozotocin-induced diabetic rats: role of neurokinin receptors

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Abstract

Background. Electrophysiological studies in the mammalian kidney have identified two major classes of sensory receptors of the afferent renal nerves; chemoreceptors (CR) and mechanoreceptors (MR). The localization of calcitonin gene-related peptide (CGRP) and substance P (SP) in these renal pelvic sensory neurons provides an anatomical basis for a possible functional interaction between the two neuropeptides and SP receptor. The present study was performed to examine the possible changes in the responsiveness of renal sensory SP and CGRP receptors in rats with streptozotocin (STZ)-induced diabetes mellitus. Due to the crucial role of renal pelvic SP and CGRP receptors in the activation of renal sensory neurons by various stimuli, we examined whether the responsiveness of MR or CR activation and the dorsal root ganglia content of neuropeptides and neurokinin 1 receptors (NK1R) were altered in diabetic rats compared with non-diabetic rats.

Methods. Afferent renal nerve activity (ARNA) was recorded from the peripheral portion of the cut end of one renal nerve branch placed on a bipolar silver wire electrode. T13 dorsal root ganglia (DRG) immunoreactivity was performed to NK1R, SP and CGRP.

Results. The results of the current study confirmed that the stimulation of renal MR and CR elicited a renorenal reflex response, and that the renal pelvic administration of SP and CGRP increased ipsilateral ARNA and contralateral urinary sodium excretion with no changes in arterial pressure. We also found a decrease in NK1R expression followed by an increase in SP and CGRP levels in the DRG of diabetic rats. The ARNA response, produced by renal pelvic MR and CR stimulation, was found to be significantly attenuated in the STZ-induced diabetic model.

Conclusions. These data may indicate a compensatory synthesis and/or abnormal axonal delivery of neurokinins from the cell body to synaptic portions of the neuron as the underlying reason for attenuated ARNA in renal sensory neurons of diabetic rats.

Keywords: afferent renal nerve activity; chemoreceptors; diabetes mellitus; mechanoreceptor; neurokinin receptors

Introduction

Most renal sensory neurons, including chemoreceptors (CR) and mechanoreceptors (MR), located in the vascular and ureteropelvic portions of the kidney may reflect the physical and chemical status of the kidney, which in turn trigger afferent renal nerve activity (ARNA) and change the contralateral natriuresis, the renorenal reflex [1–6]. Renal pelvic sensory neurons contain substance P and/or calcitonin gene-related peptide (CGRP) [2,3,5,7]. The increase in ARNA following the administration of either substance P [8] or CGRP [9] into the renal pelvis is blocked by substance P and CGRP receptor antagonists, respectively, thus confirming the presence of both receptors in the renal pelvic area. However, only substance P receptors are involved in the stimulation of renal MR and CR since a substance P receptor antagonist [8], but not a CGRP receptor antagonist [9,10], has been found to block the ARNA response to increased renal pelvic pressure or chemical stimuli. Activation of the renorenal reflex mechanism by increases in renal pelvic pressure, associated with increased urine flow, may facilitate the excretion of an increased sodium load by decreasing sympathetic effenter renal nerve activity (ERNA). 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 that lack intact afferent renal innervation [11].

Diabetic neuropathy is a serious and common complication in both type 1 and type 2 diabetes and encompasses multiple organs [12]. This pathology is multifactorial and has been attributed to vasculature diseases, leading to nerve ischaemia and/or a combination of metabolic defects associated with increased influx of glucose through the aldose reductase pathway [13]. There is a considerable evi-
dence for impaired sensitivity of carotid and aortic baroreceptor reflexes and cardiac chemoreceptor reflexes in type 1 diabetes mellitus in both patients and streptozotocin (STZ)-treated rats [14–17]. Likewise, decreased responsiveness of renal mechanosensory nerves occurs during volume expansion in STZ rats [18]. The impaired renal nerve reflexes in these rats may contribute to the altered homeostatic regulation of arterial pressure and sodium balance in STZ rats. Alterations in axonal neuropeptide content have been reported in various organs, including the gastrointestinal tract, skin, urogenital tract, blood vessels, spinal cord and brain in STZ-induced diabetes model, although the changes described do not follow a predictable pattern. Thus, levels of particular peptides have been reported to increase, decrease or remain unchanged, depending on the innervation of the target tissue.

The present study was performed to examine the possible changes in the responsiveness of renal sensory SP and CGRP receptors in rats with streptozotocin-induced diabetes mellitus. Due to the crucial role of renal pelvic SP and CGRP receptors in the activation of renal sensory neurons by various stimuli [1,8–11], we examined whether the responsiveness of MR or CR activation and the dorsal root ganglia content of neuropeptides and neurokinin 1 (NK1) receptors (NK1R) were altered in diabetic rats compared with non-diabetic (control) rats.

Materials and methods

Experiments were performed on male Wistar–UNIB/Hannover rats weighing 250–275 g (mean weight, 263 ± 4 g). The general guidelines established by the Brazilian College of Animal Experimentation (COBEA) were followed throughout the study.

STZ-induced diabetes

Diabetes (STZ-diabetes) was induced by a single intravenous tail vein injection of streptozotocin (STZ 60 mg/kg; Sigma Chemical Co., St Louis, MO, USA) dissolved in cold 0.1 M sodium citrate buffer (pH 4.5). The onset of diabetes was associated with polydipsia, polyuria, glucosuria and a decrease in body weight, followed by a second load of the same volume 1 h later. Thirty minutes after the second load (control period), spontaneously voided urine was collected over four periods of 30 min into a graduated centrifuge tube. At the end of the experiment, the animals were anaesthetized with sodium pentobarbital; blood was drawn by cardiac puncture, and urine and plasma samples were taken for analysis [19,20].

Recording of ARNA

ARNAs were recorded from the peripheral portion of the cut end of one renal nerve branch placed on a bipolar silver wire electrode. ARNA was integrated over 1-s intervals, the unit of measure being microvolts. ARNA was expressed as a percentage of its baseline value during the control period. With the aid of a dissecting microscope (Nikon, type 102), one renal nerve branch was isolated between the angle of the aorta and the left renal artery. Recordings from multi-fibre preparations were obtained by placing the renal nerve on a bipolar silver wire electrode fixed to the renal nerve with liquid polymerized resin (President, Contêne, AG, Switzerland). A high-impedance probe (Grass H8P11) carried the signals to a bandpass amplifier (Grass P11) with high and low frequency cutoffs at 3000 and 30 Hz, respectively. The signals were amplified 20000 times. The output of the bandpass filter was fed to an oscilloscope (Tektronix, TDS 340) and to a resetting voltage integrator (Grass 7P10), where the renal nerve activity was determined as the integrate (in microvolts) of the signal, U(t), during 1 s, i.e. as ∫ U(t)dt/1 s. Since the signal depended on the number of nerve fibres connected to the electrode, the nerve activity during each experimental period was expressed as a percentage of that during the preceding control period [1,8–11]. Following the establishment of optimal renal nerve activity, the renal nerve was sectioned, and the distal portion was placed on the electrode for the recording of afferent renal nerve activity. Background non-neural activity, which was assessed by crushing the decentralized renal nerve bundle peripheral to the recording electrode, was subtracted from all values of renal nerve activity.

Experimental procedures

Approximately 90 min elapsed between the end of surgery and the start of the experimental protocol. The effect of perfusing the renal pelvis with SP, CGRP, KCl and 0.9 M NaCl on renal sensory activity and natriuresis was examined in five groups of rats as follows:

- **Group 1:** stimulation of renal MR by increasing the ureteral pressure
- **Group 2:** SP-dose–response curve
- **Group 3:** CGRP-dose–response curve

To characterize the basal renal function, creatinine and lithium clearance were performed in additional conscious, unrestrained control (Co) and 4-week STZ-treated diabetic rats. Briefly, 14 h before the renal test, 60 μmol/L LiCl 100 g body weight was given by gavage. After an overnight fast, each animal received a load of tap water by gavage (5% of the body weight), followed by a second load of the same volume 1 h later. Thirty minutes after the second load (control period), spontaneously voided urine was collected over four periods of 30 min into a graduated centrifuge tube. At the end of the experiment, the animals were anaesthetized with sodium pentobarbital; blood was drawn by cardiac puncture, and urine and plasma samples were taken for analysis [19,20].

**Group 1: stimulation of renal MR by increasing the ureteral pressure**

Three 10-min control, 5-min experimental and 10-min recovery periods separated by 20-min intervals were performed in control (n = 7) and STZ-diabetic (n = 7) rats. During the experimental periods, the ureteral pelvic perfusion pressure was increased to 5, 10 and 20 mmHg (in a random order). The renal pelvis was continuously perfused with 0.15 M NaCl at 20 μL/min throughout the experiment.

**Group 2: SP-dose–response curve**

The renal pelvis of control (n = 7) and STZ-diabetic (n = 7) rats was perfused with 0.15 M NaCl, the vehicle for SP, at 20 μL/min throughout the experiment, as previously described. Four 5-min control, experimental and recovery periods were separated by 20-min intervals. The renal pelvis was perfused throughout the experimental period with vehicle (0.15 M NaCl) except during the first 2.5 min of each experimental period, when the renal perfusion was switched from the vehicle to SP at 0.62, 2.48, 9.92 or 39.68 μM (random order); the total volume of administered SP was 50 μL.

**Group 3: CGRP-dose–response curve**

The renal pelvis of control and STZ-diabetic rats was perfused as described above. CGRP (n = 7) was administered into the renal pelvis at...
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20 µL/min (total volume administered = 50 µL). Four 5-min control, experimental and recovery periods were separated by 20-min intervals. The renal pelvis was perfused throughout the experiment (n = 12) with vehicle (0.15 M NaCl) except during the first 2.5 min of each experimental period, when the renal perfusate was switched from the vehicle to CGRP at 0.0026, 0.026, 0.26 or 2.6 µM (random order); the total volume of administered CGRP was 50 µL.

Group 4: stimulation of renal CR by renal pelvic perfusion with 0.9 M NaCl
In this group, the renal CR of control (n = 7) and STZ-diabetic (n = 7) rats were stimulated by exposure to two 5-min control, experimental and recovery periods that were separated by 20-min intervals. During the experimental periods, the renal pelvic perfusate was switched from vehicle solution (0.15 M NaCl) to 0.9 M NaCl, added to the renal pelvic perfusate (n = 7).

Group 5: dose–response curve for KCl
Control and STZ-diabetic rats (n = 7 for each group) were subjected to three 10-min control, 5-min experimental and 10-min recovery periods, separated by 10-min intervals. During the first part of the experiment, the renal pelvis was perfused with 10 M NaCl, and during the second part, KCl (31.25, 62.5 or 125 mM) was added to the renal pelvic perfusate in a random order.

Immunofluorescence for NK, R, SP and CGRP
Control (n = 3) and STZ-diabetic rats (n = 3) were anesthetized and perfused via the left carotid artery with saline containing 2% heparin for 5 min under constant pressure. This was followed by perfusion with 0.1 M phosphate buffer (PB; pH 7.4) containing 4% (w/v) paraformaldehyde and 0.1 M sucrose. After perfusion, the left dorsal root ganglion (T13) was immediately removed and placed in the same fixative for 1 h, followed by PBS containing 0.1% glycine for 1 h and PBS containing 15% (w/v) sucrose overnight. Subsequently, the tissue was placed in OCT compound cryoprotector (Tissue-tech®), freeze-thawed in liquid nitrogen and cut in serial sections (10 µm thick) on a Leica cryostat at −25°C, followed by mounting on silane-coated slides. For immunohistochemistry, the sections were incubated sequentially with: (i) phosphate-buffered saline (PBS) containing 8% fetal calf serum, 2% normal goat serum, 2% milk and 2.5% bovine serum albumin (BSA) for 45 min to minimize non-specific reactions, (ii) rabbit anti-NK, R antisera (1:50 dilution; Sigma), rabbit anti-CGRP antisera (1:250 dilution; Sigma) or goat anti-SP antisera (1:1000; Santa Cruz), at 4°C overnight, and (iii) goat anti-rabbit CY2-labelled antibody (1:600 dilution; Jackson ImmunoResearch) or rabbit anti-goat CY3-labelled antibody (1:1000 dilution; Jackson Immunoresearch) for 2 h at room temperature. After incubation, the sections were rinsed in 0.1 M PBS and cover-slipped with Vectashield anti-fading medium (Vector). The sections were examined with a confocal laser scanning microscope (CLSM, LSM510 ZEISS) using laser beams of 543 and 488 nm (and UV) and appropriate emission filters for CY3 (590–610 nm) and CY2 (510–525 nm) (and DAPI). Digital images were captured using specific software (LSM; Zeiss) and were printed on a colour printer. No immunoreactivity was seen in control experiments in which one of the primary antibodies was omitted [20,21].

Chemical analyses
Plasma and urine sodium, potassium and lithium concentration were measured by flame photometry (Micronal, B262, São Paulo, Brazil), while the creatinine concentrations was determined spectrophotometrically (Instruments Laboratory, Genesys V, Lexington, MA, USA). Plasma glucose concentration was measured by an enzymatic method (Labtest, Campinas, SP, Brazil).

Drugs
Substance P and CGRP were purchased from Sigma Chemical Co. (St Louis, MO, USA) and were dissolved in 0.15 M NaCl.

Calculations and statistical analysis
Left (ipsilateral) ARNA, systemic haemodynamic and right (contralateral) urinary sodium excretion were measured and averaged over each period. In control and diabetic rats, the increase in ARNA produced by SP, CGRP, 0.9 M NaCl or 31.25, 62.5 and 125 mM KCl and increased renal pelvic pressure remained relatively constant throughout the experiments. Therefore, ARNA was averaged over each period and expressed as a percentage of the control period in Group 1, 4 and 5. The peak values of ipsilateral ARNA were measured during the administration of SP and CGRP Friedman two-way analysis of variance, shortcut analysis of variance and the Wilcoxon matched-pairs signed-rank test were used to compare the results.

Creatinine clearance was used to estimate glomerular filtration rate (GFR), and lithium clearance (CiL) was used to assess proximal tubule output [19,20]. Fractional sodium excretion (FENa) was calculated as CNa/Ccr, where CNa is the sodium clearance, and Ccr is the creatinine clearance. The fractional proximal (FEFPNa) and post-proximal (FEPPNa) sodium excretion were calculated as CFPNa/Ccr × 100 and CPPNa/Ccr × 100, respectively.

Renal parameter responses were calculated as the area under the curve versus time (AUC in Δ% min−1), where renal data were expressed as a percentage of its baseline value during the 30-min control period preceding each experimental interval. Statistical analysis of the clearance data was performed using Student’s-test. P ≤ 0.05 was taken to indicate statistical significance.

Results
The results are reported as means ± SD per 100 g body weight. The initial body weights of selected rats were similar in the two groups before STZ or vehicle injection. Four weeks after treatment with STZ, there was a smaller increase in body weight in the STZ-diabetic rats compared with control rats. There was also a significant increase in the kidney weight per 100 g of body weight in diabetic rats compared with control rats. The fasted blood glucose levels exceeded 350 mg/dL in diabetic rats. The daily urine flow was significantly higher in diabetic rats (data not shown), whereas the plasma sodium and potassium concentrations did not differ between the two groups. The basal mean arterial pressure, but not heart rate, was significantly higher in diabetic-induced rats compared with the control group (Table 1).

The renal function data obtained from additional conscious, unrestrained control (Co) and 4-week STZ-treated diabetic rats are summarized in Table 2. The glomerular filtration rate estimated by Ccr differed significantly among the groups (STZ: 331 ± 34 vs. 300 ± 25 Δ% min−1 in Co group, P = 0.032) during the studies of renal tubule sodium handling (Table 2). The results also showed an increased FENa in STZ diabetic rats (883 ± 46 Δ% min−1) when compared with Co group (925 ± 38 Δ% Min−1) (P = 0.039). The enhanced FEFPNa was accompanied by a significant increase in proximal sodium excretion (P = 0.0001).

Group 1: stimulation of renal MR by increasing the ureteral pressure
Increasing the ureteral pressure to 5, 10 and 20 mmHg in the control group of rats resulted in graded increases in ipsilateral ARNA [14.6 ± 8.7%, 49.1 ± 8.2% (P < 0.01), and 98.4 ± 15.2% (P < 0.01), respectively] and in contralateral urinary sodium excretion [7.6 ± 1.0%, 24.1 ± 3.7% (P < 0.01), and 42.4 ± 7.4% (P < 0.01), respectively]. Graded increases in ureteral pressure of the same magnitude in diabetic rats resulted in a blunted increase in ipsilateral ARNA [17.8 ± 9.7%, 31.8 ± 6.3% and 43.2 ± 18.2%, respectively] and contralateral urinary sodium excretion [7.8...
The data are reported as the mean ± SD. AUC, area under curve in min proximal (FEPNa +) and post-proximal (FEPPNa +) fractional sodium excretion and fractional potassium excretion (FEK +) of control (Co) rats compared with STZ-induced diabetic (STZ) rats.

**Group 2: dose–response curve for SP**

Figures 1 and 2 show an unaltered basal ARNA (183 ± 46 μV/s/1 s) throughout all experimental groups. In the control group, SP increased ipsilateral ARNA in a concentration-dependent fashion (Figure 1). The durations of the ARNA responses to SP (0.62–39.68 μM) were similar and averaged 36 ± 3.2 s. Mean arterial pressure and heart rate were unaltered by SP (0.62–9.92 μM). At 39.68 μM, SP significantly reduced the basal mean arterial pressure by 10 ± 3 mmHg (P < 0.05) but not the heart rate. There was a gradual increase in basal contralateral urinary sodium excretion during the experiment, from 10.2 ± 4.6% to 31.7 ± 7.1% with 0.62–39.68 μM, respectively (P < 0.05). In diabetic rats, the increase in ARNA and UNaV in response to the same concentrations of SP was significantly attenuated. In these rats, the basal blood pressure (142 ± 5.3 mmHg) and heart rate (298 ± 23 bpm) were unaltered by the different concentrations of SP.

**Group 3: dose–response curve for CGRP**

In control rats, the renal pelvic administration of CGRP (0.0026–2.6 μM) increased ipsilateral ARNA in a concentration-dependent fashion (Figure 1). The duration of the response to CGRP increased with increasing concentration, from 13 ± 3 s (0.026 μM) to 46 ± 5 s (2.6 μM). Contralateral urinary sodium excretion (Figure 2) increased to 20.5 ± 5.0% from 1.3 ± 0.5 μmol min⁻¹ g⁻¹ and by 21.7 ± 4.6% from 1.1 ± 0.3 μmol min⁻¹ g⁻¹ (both P < 0.01) in response to 0.26 and 2.6 μM CGRP, respectively. The mean arterial pressure (121 ± 10 mmHg) and heart rate (327 ± 13 bpm) were unaffected by CGRP in both groups. In diabetic rats, the increase in ARNA and UNaV in response to the same concentrations of SP was significantly attenuated (Figures 1 and 2). In these rats, the basal blood pressure (132 ± 8 mmHg) and heart rate (308 ± 22 bpm) were unaltered by the different concentrations of CGRP.

**Group 4: stimulation of renal CR by renal pelvic perfusion with 0.9 M NaCl**

In control rats, renal pelvic perfusion with 0.9 M NaCl increased the ipsilateral ARNA (51.6 ± 4.7%, P < 0.02), associated with increased but non-significant contralateral urinary sodium excretion (17 ± 8.4%, P < 0.05) (Figure 1). The ARNA responses to renal pelvic perfusion with 0.9 M NaCl were not significantly altered in diabetic rats. Likewise, the natriuretic responses to renal pelvic perfusion with 0.9 M NaCl were unaltered in control or diabetic groups. The mean arterial pressure and heart rate were unaffected by renal pelvic perfusion with 0.9 M NaCl in control (109 ± 6 mmHg; 322 ± 8 bpm) and diabetic (113 ± 5 mmHg, 307 ± 16.3 bpm) rats.

**Group 5: KCl-h-CGRP (8–37)**

Renal pelvic perfusion with KCl (31.25, 62.5 and 125 mM) caused a significant concentration–dependence increase in ARNA (27.3 ± 3.7%, P < 0.02) at 125 mM KCl (Figure 1) in the control group. That ARNA response to KCl pelvic administration in diabetic rats was unaltered (see Figure 1). The mean arterial pressure and heart rate (112 ± 6 mmHg and 308 ± 10 bpm, respectively) were unchanged by renal pelvic perfusion with different concentrations of KCl in both groups.

**Immunofluorescence**

In control DRG cells, NK<sub>1</sub>R immunoreactivity occurred predominantly in the nuclei and on the surface of cells, with only disperse and weak reactivity in the cytosol (Figure 3). This pattern was seen in the different subpopulations of neurons, which were defined based on their diameters (10–25 m for small cells, 25–37.5 m for intermediate cells and 37.5–60 m for large cells). In diabetic rats, this pattern of distribution was the opposite, with little or no nuclear staining but large, densely marked vesicles in the cytosol (Figure 3). Overall, the immunoreactivity for NK<sub>1</sub>R was weaker in diabetic compared with control rats.
The substance P body cell neuron immunoreactivity was stronger in diabetic than in control rats (Figure 4). In control rats, the subcellular distribution of SP was predominantly and heavily distributed in the cytosol. In T13 DRG of diabetic rats, SP immunoreactivity was significantly greater on the cell surface (Figure 4). All DRG cells showed a similar pattern of localization.

CGRP neuronal immunoreactivity occurred predominantly in the cytosol of DRG cells in control rats (Figure 3). In DRG from diabetic rats, increased CGRP immunoreactivity was significantly greater on the cell surface (Figure 4). All DRG cells showed a similar pattern of localization.

CGRP neuronal immunoreactivity occurred predominantly in the cytosol of DRG cells in control rats (Figure 3). In DRG from diabetic rats, increased CGRP immunoreactivity distribution occurs particularly in the perikaryon, and also in many cells, there was a greater immunoreactivity close to the cellular membrane (Figures 3 and 4).

**Discussion**

We and others [1,8–11,21] have previously demonstrated that neurokinins have important biological roles in the regulation of kidney function. However, to the best of our knowledge, the entire physiological significance of renal SP and CGRP receptors remains unclear, and no study has reported the participation of these peptides in the sensory neuropathy observed in the STZ diabetic model. Immunocytochemical studies have shown that most renal afferent sensory neurons from DRG are located in the renal pelvic wall [3–6], and that they contain the neurokinins SP and CGRP [3,6]. Increases in ARNA, elicited by these neurokinins, are blocked by renal pelvic perfusion with selective SP and CGRP receptor antagonists [8,9], indicating that neuropeptides activate renal sensory receptors in the pelvic region. The results of the current study confirmed that the stimulation of renal MR and CR elicited a renorenal reflex response, and that the renal pelvic administration of SP and CGRP increased ipsilateral ARNA and contralateral urinary sodium excretion with no changes in arterial pressure. Additionally, our findings suggest that the increased ARNA response produced by renal pelvic...
perfusion with SP, CGRP, 0.9 M NaCl or 31.25–125 mM KCl in non-diabetic rats was significantly attenuated in the STZ-induced diabetic model.

SP is involved in renal pelvic MR activation since a rise in renal pelvic pressure (UP) [1,8] raises the renal release of SP [22] and the level of ARNA [8,11]. On the other hand, CGRP has been suggested to be a neuromodulator of renal sensory receptors [10]. These sensory neurons may regulate their responses to different stimuli (or the same stimuli at different intensities) by altering the ratio of CGRP and SP released. In theory, the basal activity of MR could be increased because of a diabetes-associated elevation in diuresis. However, our study, in agreement with in vitro study [22], revealed in vivo no enhanced basal activity of MR and a blunted (desensitized) response to increasing UP in the hyperglycaemic STZ rat compared with control group. The present study also confirms previous findings [1,10], showing that stimulation of renal CR with 0.9 M NaCl or 31.25–125 mM KCl increased ARNA and contralateral natriuresis in a concentration-dependent manner. The threshold of activation was <61.5 mM KCl in non-diabetic rats; however, this effect did not occur in diabetic rats. The contralateral natriuresis is associated with a fall in efferent renal nerve activity and can be blocked by denervation of either kidney [1], indicating that this is a renorenal reflex-mediated response. Hypertonic NaCl increases the release of CGRP from rat isolated urinary bladder [23], and chronic treatment with capsaicin, which depletes sensory neurons of CGRP and SP [1,8,9], suppresses the ARNA response to renal pelvic perfusion with 0.9 M NaCl. On the other hand, the depolarization of sensory neurons by KCl is associated with a release of CGRP from isolated soleus and dorsal spinal cord preparations [24,25].

Fig. 2. Urinary sodium excretion responses (U\textsubscript{NaV} in %) to graded increases in ureteral pressure or, after renal pelvic administration of increasing concentrations of SP, CGRP, 31.25, 62.5 and 125 mM KCl and 0.9 M NaCl. The figure also shows the arterial pressure responses (in mmHg) during follow-up of control and diabetic (STZ) rats. *P < 0.05 vs. vehicle (Friedman two-way analysis of variance, shortcut analysis of variance and the Wilcoxon matched-pairs signed-rank test were used).
The renal sensory receptor activity reflects the hydrostatic or chemical environment in the kidney, and its potential function also lies in the initiation of reflex effects that may affect the kidney directly through its efferent innervation or indirectly through circulatory changes [26,27]. In STZ-diabetic rats, the natriuretic response to acute volume expansion is impaired, at least in part, due to a reduced decrease in ERSNA [15]. 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 rats [15,28] suggests that the decreased responsiveness of the renal mechanosensory nerves, such as those observed in our study, contributes to the impaired excretory response to acute volume expansion in these rats. Thus, in the current study, the impaired MR and CR response may indicate that abnormal renal reflex in STZ-diabetic rats could be attributable to the desensitization of renal afferent activity.

Fig. 3. Immunofluorescence for NK1, SP and CGRP in T13 DRG of control (Co) and diabetic (STZ) rats.

Fig. 4. Detail of medium-sized neurons in T13 DRG, showing the distribution pattern of SP and CGRP in control (Co) and diabetic (STZ) rats.
As have been shown, early-onset diabetes in the STZ rats is characterized by increased renal blood flow, glomerular filtration rate, glucosuria, polyuria and natriuresis [6,22], all factors that could alter the responsiveness of the renal mechano-sensory nerves. In the present study, we confirm natriuretic responses in STZ-induced diabetic rats that occurred by decreasing proximal tubule Na+ re-absorption, associated with an enhanced Ccr (Table 2) and proportionate to the Na+ filtered load. A study in type 1 diabetic patients suggests an important role for hyperglycaemia stimulating the renin – angiotensin system (RAS) [29]. Also, there is evidence for a role of increased angiotensin II (AngII) activity in STZ-induced neuropathy. Systemic administration of AngII antagonists improved sensory nerve conduction velocity possibly by increasing neural blood flow [30,31]. In the present study, performed in rats with early-stage type 1 diabetes, we cannot exclude the possibility that increased RAS in the STZ diabetic renal pelvic wall [18] may have impaired the neuropeptide activation of sensory renal nerve receptors. Glucosuria, a defining feature of diabetes, results in urinary osmotic salt and water losses. Previous study by Song et al. (2003) has shown that the kidney adapts to sodium and water losses associated with STZ-diabetes via upregulation of both sodium and water transport proteins. These findings are critical for the maintenance of extracellular fluid volume in the face of large osmotically driven fluid losses. In the same study [32], the authors exclude the potential toxicity of STZ (65 mg/kg b.w.), with regard to all parameters measured, including urinary volume, osmolality, kidney size, and transporter and channel abundances, and the STZ-treated rats were similar to the vehicle-treated rats. On the other hand, studies in euglycaemic normal rats have shown that a urinary hypertonic mannitol concentration of 1.8 M has no effect on the activity of renal pelvic mechano-sensory nerves [33], suggesting that the impaired responsiveness of renal MR or CR [18] are not related to hyperglycaemia levels proportionate to the filtered load and consequently higher urinary glucose concentration.

Diabetic neuropathy is a symmetric polyneuropathy characterized by a wide range of peripheral neuronal deficits, including reduced motor nerve conduction velocity, impaired regenerative capabilities [34], axonal shrinkage, in association with reduced neuropeptide delivery [35], and defective axon transport mechanisms [36–38]. However, no signs of morphological damage to rat peripheral nerves are apparent in diabetic rat models of a short duration [18,39]. Diabetes-induced alterations in axonal neuropeptide content have been reported in various organs, including the gastrointestinal tract, skin, urogenital tract, blood vessels, spinal cord and brain, although the changes described do not follow a predictable pattern. Thus, levels of particular peptides have been reported to increase, decrease or remain unchanged, depending on the target tissue innervation. We investigated the neuropeptide DRG body cell immunoreactivity of SP and CGRP, and NK1R in normal and diabetic rats to determine whether there was any change in their pattern of distribution. In view of the result of the current study, the higher DRG immunoreactivity of SP and CGRP in STZ rats may argue against the idea that the decreased responsiveness of the renal sensory nerves is due to reduced substance P and CGRP contents in this experimental model. 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 [40,41].

Previous studies in diabetic rats have demonstrated a reduced expression of neurofilament in sensory neurons [42], reduced axonal transport of neurofilament in motor axons [35], loss of neurofilament in distal nerves [43], and abnormal neurofilament phosphorylation in the spinal cord and sciatic nerve [44,45]. Based on these data, we may hypothesize that neurofilament abnormalities in sensory renal neurons of diabetic rats may interfere with the axonal transport and synaptic access of neuropeptides, thereby leading to distal sensory axonopathy, including in the kidney. As shown here, the expressions of SP and CGRP were enhanced in the perikarya of DRG neurons in diabetic rats, and these results may indicate the involvement of a supposed defect in the axonal transport of these neuropeptides.

Since neurons with the longest axons are the most vulnerable in diabetes mellitus, neuropeptide-mediated pathologies have been extensively studied in the sciatic nerve and the corresponding L4 and L5 DRG. The depletion of substance P and CGRP in the sciatic nerve endings of rats with STZ-induced diabetes has been demonstrated [37], and can be substantially attenuated by intensive treatment with insulin [36,38,46]. However, in contrast to our findings, the neuropeptide deficiency in the sciatic nerve is accompanied by a reduction in neuropeptide expression [47,48] in the DRG. The anterograde transport of peptides is also reduced in this model of diabetes [35,49], and an increase in the content of SP and CGRP in perikarya may indicate their accumulation in neurons. The decreased levels of NK1R in the body cells of pelvic neuron, shown in our study, may suggest a reduction in the delivery of anterogradely transported SP and CGRP from T13 DRG to the renal nerve endings, rather than a reduction in the synthesis of these neuropeptides. This mechanism may play, at least in part, a role in the desensitization response to MR and CR stimuli in diabetic rats. However, to confirm this hypothesis, it would be necessary to measure the renal peptide levels in diabetic rats, as well as the axonal transport, as carried out by Marfurt and Echtenkamp [4] in the eyes of 3-month-old diabetic rats; these authors found no differences in the SP and CGRP contents in control and diabetic corneas.

In conclusion, in the present study, we found a pronounced decrease in NK1R expression, followed by an increase in SP and CGRP levels in the perikarya of DRG in diabetic rats. These findings may indicate a compensatory synthesis and/or abnormal axonal delivery of neurokinins from the cell body to synaptic portions of the neuron as the underlying reason for attenuated ARNA in renal sensory neurons of diabetic rats. Based on these findings, we hypothesize that impaired renal sensory endings affect the fluid homeostasis by an abnormal renorenal reflex, consequently leading to the development of arterial hypertension, in diabetic rats.
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Acknowledgements. The authors thank Adriana R. M. Créto for expert technical assistance. This research was partially supported by CNPq (No.500868/91-3), CAPES and FAPESP (06/52431-1).

Conflict of interest statement. None declared.

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Received for publication: 22.3.10; Accepted in revised form: 28.7.10

Prevention of accelerated atherosclerosis by AT1 receptor blockade in experimental renal failure

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Abstract

Background. The mechanisms of uraemia-induced atherosclerosis have not been fully delineated. The aims of this study were (i) to investigate the extent and the phenotype of atherosclerosis, including the activation of local renin–angiotensin system (RAS), in a mouse model of mild uraemia and (ii) to determine the effects of angiotensin II type1 (AT1) receptor blockade on the uraemic atherosclerosis, clarifying the mechanisms of its action.

Methods. Mild uraemia was induced by 5/6 nephrectomy in 8-week-old apo E-deficient mice (apoE-KO). After nephrectomy, the animals received either treatment with candesartan or no treatment for 12-weeks. Sham-operated apoE-KO mice were used as controls.

Results. Uraemia led to a two-fold increase in aortic plaque area. This was associated with a significant upregulation of aortic angiotensin-converting enzyme (ACE), AT1 receptor, connective tissue growth factor (CTGF), monocyte chemoattractant protein (MCP)-1, and vascular cell adhesion molecule (VCAM)-1. Candesartan significantly reduced aortic atherosclerosis, prevented the upregulation of the uraemia-induced genes and led to changes predicting greater stability of the plaques, without influencing blood pressure or serum lipids.

Conclusions. This study indicates that uraemia leads to an acceleration of aortic atherosclerosis. The upregulation of aortic RAS and the reduced atherosclerosis following AT1 receptor blocker treatment highlights the pivotal role of the local RAS in the development and acceleration of atherosclerosis in uraemia.

Keywords: angiotensin; AT1 receptor blockers; atherosclerosis; uraemia

Introduction

Patients with chronic kidney disease (CKD) are at greater risk of developing cardiovascular disease (CVD) than subjects with normal renal function, and in these patients, the risk for CVD seems to be much greater than the risk for renal replacement therapy [1]. Lindner and colleagues first hypothesized that the high rate of myocardial infarction in haemodialysis patients was due to an acceleration of atherosclerosis [2], which is now widely recognized as the primary cause of increased cardiovascular morbidity and mortality in this group of patients [3].

Uraemic patients have a high prevalence of classic risk factors for plaque formation, such as advanced age, hypertension, glucose intolerance and dyslipidaemia, but this does not seem to fully explain their accelerated atherosclerosis. As an instance, statin treatment in type-2 diabetes patients with end-stage renal disease has...