Increased renal sympathetic nerve activity leads to hypertension and renal dysfunction in offspring from diabetic mothers

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HYPERTENSION IS A MAJOR RISK factor for heart disease, stroke, and kidney disease (41). Epidemiological and experimental studies have shown that hypertension may be programmed during the prenatal period (33, 34). Fetal programming occurs in response to environmental insults in early life, particularly in the uterus, and may cause permanent structural and biochemical changes in the body, resulting in modifications that later in life can become threats to health (7).

In the last decade, several studies have demonstrated that sustained exposure of the fetus to excess glucose increases the risk of intrauterine death, premature birth, perinatal mortality, and congenital malformations (39, 43). Offspring of diabetic mothers have an increased risk of cardiovascular disease and diabetes in adulthood (2, 13, 42). A recent meta-analysis concluded that offspring of diabetic mothers have higher systolic blood pressure than controls (1).

Previous studies from our laboratory have shown that maternal diabetes promotes systemic hypertension and glomerular hypertrophy in the offspring of diabetic rats, which contribute to the progression of renal disease (11, 40, 52). The hypertensive status was accompanied by reduced glomerular filtration rate (GFR) and sodium retention (51) and also by an impaired vasodilatory response to acetylcholine and bradykinin (52). The development of hypertension and glomerular hypertrophy was blunted by administration of L-arginine (11).

The role of renal sympathetic nerve activity (rSNA) in generating and maintaining hypertension became evident in studies which showed that renal denervation reduced blood pressure in various experimental models of hypertension (4, 24, 66). In addition, renal denervation also reduced blood pressure in patients with drug-resistant hypertension (21, 61).

Increased rSNA can contribute to the genesis of hypertension both directly by increasing tubular water and sodium reabsorption and indirectly by increasing the renal secretion of renin, which activates the renin-angiotensin system resulting in increased vascular resistance and reduced GFR (17).

Considering that increasing evidences have pointed to renal sympathetic hyperactivity as a causal mechanism in the development and maintenance of hypertension in its many forms, and that so far, there is no information about the role of rSNA in hypertension and renal dysfunction in the changes observed in offspring of diabetic mothers, the present study was conducted to evaluate the role of rSNA in renal function and blood pressure in this experimental model. Our data show that offspring from diabetic mothers have renal sympathetic hyperactivity and impaired baroreflex control of rSNA. These changes are probably related to hypertension and renal dysfunction observed in this experimental model.

MATERIALS AND METHODS

All procedures used in this study were in accordance with the “Guide for Care and Use of Laboratory Animals” published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and approved by the Research Ethics Committee of the Federal University of São Paulo (process No. 10162). The role of rSNA on renal function and blood pressure in offspring of diabetic mothers was studied in two steps: 1) analysis of the effect of renal denervation on blood pressure and renal function in offspring from diabetic and control mother rats and 2) analysis of renal nerve activity in offspring from diabetic and control mother rats.
Offspring of Diabetic Dams

Female Wistar rats (200–240 g) from our colony (Centro de Desenvolvimento de Modelos Experimentais) were maintained in a room at 22 ± 1°C with a 12:12-h light-dark cycle with food and water freely available. They received an intraperitoneal injection of streptozotocin (60 mg/kg body wt; Sigma) to induce diabetes mellitus. A small blood sample was collected 48 h later from a small puncture of the tail. Blood glucose was measured with an Accu-Chek Advantage glucometer (Roche Diagnostics, Mannheim, Germany). Rats with glucose levels above 250 mg/dl were considered diabetic. Control rats did not receive streptozotocin or vehicle injection. Control and diabetic rats were caged with a male rat and housed individually after mating. Maintenance of the diabetic status was confirmed weekly during pregnancy. Rats were discarded if blood glucose fell below 250 mg/ml. After birth, up to six male offspring were left with the mother until they were 28 days old (52). Of these, one was randomly chosen to be subjected to renal denervation, and one to be subjected to sham surgery. The others were used in another study. The same procedure was followed for offspring of nondiabetic dams.

Effect of Renal Denervation on Blood Pressure and Renal Function

Caudal systolic pressure was measured in 3-mo-old male offspring from control and diabetic dams. Control and diabetic offspring were randomly distributed in four groups: 1) offspring from control mother sham (OC-sham); 2) offspring from control mother denervated (OC-dn); 3) offspring from diabetic mother sham (OD-sham) and 4) offspring from diabetic mother denervated (OD-dn). Following grouping, rats were denervated or sham-operated. Ten days were allowed for recovery from the surgery, and then systolic pressure was measured again. Two weeks after sham or denervation surgery, the animals were prepared for the evaluation of renal function.

Indirect measurement of arterial blood pressure. Systolic blood pressure was determined in conscious rats with a tail cuff method (ITTC Life Science). The rats were habituated with the procedure from the ages of 70–75 days old. For this procedure, the rats were placed in a restrainer and maintained at 33–34°C. The cuff was placed at the base of the tail and inflated. After this maneuver was performed five to six times, the rats got used to the procedure. We obtained three measurements in sequence, and the mean value of these measurements was considered the value of the blood pressure. The measurements of blood pressure were obtained when rats were 3 mo old. The same procedure was performed 10–12 days after denervation or sham surgery. The measurements were not performed in a blinded fashion, but they were done by a computer system without the interference of the experimenter.

Renal denervation. Renal denervation was performed as described previously (38). Briefly, the rats were anesthetized with ketamine and xylazine (40 and 20 mg/kg ip, respectively; Vetbrands, Jacaref, Brazil) and the renal pedicle was exposed through a dorsal incision. The renal artery, vein, and nerve were identified under a surgical microscope. The nerve was isolated and sectioned. A section of the renal artery was wrapped for 5 min with cotton soaked in a solution of 10% phenol in absolute ethanol. The procedure was repeated for the renal vein. The procedure was performed bilaterally. The muscle walls were closed with absorbable suture, and the skin was closed with silk suture. In sham-operated animals, the renal nerves were visualized but not touched.

Evaluation of renal function. Renal function was studied in the offspring (groups: OC-sham, OC-dn, OD-sham, and OD-dn) 2 wk after sham or denervation surgery. The animals were anesthetized with sodium thiopental (30 mg/kg ip) and placed on a warmed table to maintain body temperature at 37°C. Tracheotomy was performed, followed by insertion of polyethylene catheters into the carotid artery for blood sampling and into the jugular vein for the infusion of saline containing mannitol 3%. A catheter was inserted into the bladder for urine collection. A 30-min infusion was made with saline containing 3% mannitol at a rate of 0.1 ml/min. This was followed by a priming injection of 1 ml of saline containing inulin (300 mg/kg) and sodium paraaminomipropionate (PAH; 6.66 mg/kg). An infusion of 0.1 ml/min containing NaCl 0.9%, inulin (5 mg·min⁻¹·kg⁻¹), PAH (1.33 mg·min⁻¹·kg⁻¹), and 3% mannitol was started that lasted until the end of the experiment. Four urine collections were made, each 30 min, starting 30 min after injection of the priming solution. Mannitol was added to increase urinary flow. This is a technique that has been used by our group and others (11, 31, 40, 52). Plasma and urine inulin and PAH concentrations were measured by colorimetry for estimation of GFR (23) and renal plasma flow (59). Sodium concentration was measured in samples of urine and plasma collected during clearance experiments. The amount of fluid infused up to the first urine collection was 7 ml, and this would be expected to increase extracellular fluid volume by < 10%. Plasma and urine Na⁺ concentration were measured with a flame photometer (model 910; Analyser).

After completion of renal function measurements in some animals, the left and right kidney were removed for analysis of tyrosine hydroxylase by immunohistochemistry and Western blot analysis. The expression of renin in renal tissue was evaluated by Western blot analysis.

Western blot analysis. The kidneys were homogenized in a buffer containing 100 mM Tris, pH 7.6, 10 mM EDTA, 10% SDS, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, and 10 mM sodium orthovanadate. Insoluble material was removed by centrifugation at 15,000 g at 4°C for 20 min. Protein content of the supernatant was measured by the colorimetric method (Bradford). Protein samples (50 μg each) were separated on 8% polyacrylamide gel. Proteins were transferred to nitrocellulose membrane by electrohoresis for 90 min at 120 V. Membranes were incubated for 1–3 h at room temperature in blocking buffer (5% nonfat dry milk, 10 mM Tris, 150 mM NaCl, 0.02% Tween). Membranes were then incubated overnight at 4°C with a 1:1,000 dilution of anti-tyrosine-hydroxylase antibody (cat. no. MAB318; Millipore) or with a 1:500 dilution of anti-renin antibody (cat. no. SC-133145; Santa Cruz Biotechnology, Santa Cruz, CA). Subsequently, the membranes were incubated for 60 min at room temperature in buffer (1% nonfat dry milk, 10 mM Tris, 150 mM NaCl, and 0.02% Tween 20) containing a 1:10,000 dilution of a secondary antibody conjugated to horseradish peroxidase (GE Healthcare, Buckinghamshire, UK). Immunoreactivity was visualized with enhanced chemiluminescence reagents (GE Healthcare Buckinghamshire, UK), and the membranes were exposed to preflashed Kodak XAR film (Eastman Kodak, Rochester, NY). Band intensities were quantified by optical densitometry (Scion Image Software, Frederick, MD) (46).

Immunohistochemistry for tyrosine hydroxylase. Kidneys were dissected out rapidly, cleaned of connective tissue, sectioned longitudinally, and immersed in Bouin’s liquid for 24 h. The kidneys were dehydrated, embedded in paraffin, and cut with a microtome in 7-μm sections. Sections were incubated for 30 min at 95°C in 10 mM citric acid buffer (pH = 6) for antigen retrieval. The sections were incubated overnight at 4°C with anti-tyrosine-hydroxylase antibody (1: 100, cat. no. MAB318; Millipore). The reaction product was determined with a streptavidin peroxidase complex (LSAB System HRP; DAKO, Carpinteria, CA) (46).

Renal Sympathetic Nerve Activity and Blood Pressure in Offspring from Diabetic Dams

To perform this part of the experimental protocol, additional litters were raised. The rats used for this experiment were all from different litters. The experiments were performed in 3-mo-old rats.

Direct measurement of arterial pressure in conscious rats. Offspring from control or diabetic mothers were anesthetized with ketamine and xylazine (40 mg/kg and 20 mg/kg ip, respectively; Vetbrands, Jacaref, Brazil). Polyethylene catheters were inserted into the femoral vein and artery. The catheters were led subcutaneously to the
renal function in the control group. Parameters were similar to control. Denervation did not change compared with other groups. In group OD-dn, renal function fractional excretion of sodium were observed in OD-sham Reduced GFR, urine flow, urinary sodium excretion, and same group of animals. Values of blood pressure in sham-operated rats in OC. Values of blood pressure in sham-operated rats depended on background noise that was determined for each experiment.

For the analysis of arterial baroreceptor control of rSNA, blood pressure was altered by infusions of sodium nitroprusside and phenylephrine (Sigma-Aldrich) (22). Phenylephrine and sodium nitroprusside were infused with a syringe pump for 1 min at rates that increased linearly from 0 to 20 μg/min (ramp infusion). Subsequent infusions were made only after blood pressure returned to baseline, but at least 5 min was allowed between infusions. Values of matching MAP variations (ΔMAP from 10 to 40 mmHg) with reflex rSNA (ΔrSNA) responses were plotted separately for each vasoactive drug to create linear regression curves of baroreceptor function for each group, and their slopes (spikes·s⁻¹·mmHg⁻¹) were compared to evaluate changes in baroreflex gain. The steeper the slope of this distribution, the more sensitive is overall baroreflex function, with respect of control of rSNA (9).

Statistical Analysis

The results are shown as means ± SE. Data were analyzed by a paired or unpaired Student’s t-test, or by one-way ANOVA, followed by the Bonferroni posttest. The level of statistical significance used was P ≤ 0.05.

RESULTS

Effect of Renal Denervation on Blood Pressure and Renal Function

Systolic blood pressure measured at 3 mo was higher in offspring from diabetic dams (OD) than in offspring from control dams (OC) (OD: 146 ± 1.6 mmHg, n = 19; OC: 117 ± 1.4 mmHg, n = 18, P < 0.0001). Values of systolic blood pressure obtained after the surgeries are shown in Fig. 1. Renal denervation (dn) reduced blood pressure in OD, but did not alter pressure in OC. Values of blood pressure in sham-operated rats were similar to the values obtained before the surgeries for the same group of animals.

The parameters of renal function are shown in Table 1. Reduced GFR, urine flow, urinary sodium excretion, and fractional excretion of sodium were observed in OD-sham compared with other groups. In group OD-dn, renal function parameters were similar to control. Denervation did not change renal function in the control group.

Immunohistochemistry showed the presence of tyrosine hydroxylase in the kidneys of rats with intact renal nerves, but not in rats subjected to renal denervation. Western blot analysis showed that renal tyrosine hydroxylase content was reduced
after renal denervation confirming the efficacy of the denervation surgery (Fig. 2). In Western blot analysis, we also observed that denervation did not change significantly the renal content of renin in the studied groups (OC-sham: 0.68 ± 0.11; OC-dn: 0.72 ± 0.06; OD-sham: 0.77 ± 0.02; OD-dn: 0.92 ± 0.07 arbitrary units; n = 4).

Renal Sympathetic Nerve Activity and Blood Pressure in Offspring from Diabetic Dams

Direct measurement of blood pressure confirmed that blood pressure was increased in the OD group (Fig. 3A). Heart rate was higher in the OD group (Fig. 3B). Basal rSNA was increased in the OD group and was seen both when renal nerve activity was expressed as spikes per second (Fig. 3C) and when it was expressed as percentage of maximum activation (Fig. 3D).

Figure 4A shows representative traces of changes in rSNA induced by the infusion of vasodilator and vasoconstrictor drugs. As shown in Fig. 4B, the rSNA baroreceptor sensitivity in the OD group was significantly reduced for the reflex responses induced by administration of either phenylephrine (baroreceptor loading) or sodium nitroprusside (baroreceptor unloading).

DISCUSSION

The main findings of the present study were 1) renal denervation was able to restore renal function and blood pressure to normal values in offspring from diabetic mothers, but it did not interfere in these variables in control rats; 2) basal renal nerve activity was increased in offspring of diabetic dams, and the arterial baroreceptor control of renal nerve activity was impaired. Our data suggest that the hypertension and renal dysfunction in offspring of diabetic dams is associated with increased renal nerve activity.

The hypertension and renal dysfunction in offspring of diabetic dams observed by us confirms several previous studies.

Table 1. Parameters of renal function in the studied groups

<table>
<thead>
<tr>
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<th>Offspring from Control Mothers</th>
<th>Offspring from Diabetic Mothers</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Denervated</td>
</tr>
<tr>
<td>Urinary flow, ml·min⁻¹·kg⁻¹</td>
<td>0.12 ± 0.01</td>
<td>0.13 ± 0.01</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·kg⁻¹</td>
<td>6.6 ± 0.2</td>
<td>6.8 ± 0.1</td>
</tr>
<tr>
<td>RPF, ml·min⁻¹·kg⁻¹</td>
<td>20.2 ± 0.7</td>
<td>20.3 ± 0.5</td>
</tr>
<tr>
<td>FENa⁺, %</td>
<td>1.1 ± 0.06</td>
<td>1.3 ± 0.09</td>
</tr>
<tr>
<td>EANa⁺, mEq·min⁻¹·kg⁻¹</td>
<td>10.3 ± 0.7</td>
<td>12.56 ± 1.03</td>
</tr>
<tr>
<td>No. of animals</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>No. of samples</td>
<td>27</td>
<td>44</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SE. Glomerular filtration rate (GFR), renal plasma flow (RPF), fractional excretion of sodium (FENa⁺), excreted amount of sodium (EANa⁺). *P < 0.05 compared with offspring from control mothers (sham), †P < 0.05 compared with offspring from diabetic mothers (sham).
(2, 11, 12, 40, 51, 52, 67). Hypertension develops early in life, before 2 mo in rats, and blood pressure increases by up to 30 mmHg. We confirmed hypertension by both direct and indirect methods (indwelling catheter and tail cuff) in nonanesthetized animals. The present study shows that glomerular filtration, urine flow, and sodium excretion are reduced in offspring of diabetic dams. We have previously shown several changes in renal function in offspring of diabetic dams. This includes reduced GFR and glomerular hypertrophy (40) and impaired ability to eliminate a sodium load (51).

Renal denervation promoted the normalization of systolic blood pressure, GFR, and urinary flow in offspring from diabetic mothers. In control animals, the denervation did not change blood pressure or renal function. These results are in accordance with the literature, which suggests that in normal conditions renal sympathetic nerve activity is low, and the denervation in this situation results in little influence on renal function and blood pressure. However, in states in which renal sympathetic nerve activity is increased, denervation has a significant effect (3, 32, 41, 45). The role of the renal nerves in setting basal level of blood pressure in normal animals has been matter of debate. In fact, the results obtained to this issue are controversial (30). This may be due, at least in part, to the diversity of methods used. In some studies, the measurements were performed in anesthetized animals a few minutes after denervation (8); in others, measurements were performed some days after denervation in anesthetized animals (26) or in awaked animals (44).

Our finding that renal denervation decreased blood pressure and restored renal function in offspring from diabetic dams is new as well as the increased basal renal nerve activity in offspring of diabetic dams. The increased renal nerve activity is related to increased renin release, increased sodium reabsorption, and reduced glomerular filtration (17). Our finding that GFR and fractional excretion of sodium were low in offspring of diabetic dams is compatible with activation of renal nerve activity in these animals, as is the reversion of these changes by renal denervation. The arterial baroreceptor regulation of renal nerve activity was also blunted in offspring of mothers with diabetes. The reduction of blood pressure induced by renal denervation may change the central mechanisms that regulate the cardiovascular system leading to changes in the reflex control of sympathetic drive to other regions of the body. Furthermore, it is well known that the renal sympathetic reflex is able to change the reflex control of circulation, and this is centrally organized in the ventrolateral medulla, a key region for the control of efferent sympathetic drive (62).

The idea that sympathetic overactivity causes the development of hypertension has been discussed since the Framingham study in 1947 (36). This hypothesis is consistent with increased renal norepinephrine spillover (20, 56) and increased firing of postganglionic sympathetic fibers in skeletal muscle in hypertensive patients (5). Increased renal sympathetic activity can lead to the development of hypertension due to a direct effect on tubular function resulting in sodium and water retention or indirectly by increasing renin release from juxtaglomerular cells (17, 18). Besides the effect of sympathetic fibers on juxtaglomerular cells, renin synthesis/secretion is also coordinated by factors regulated by the macula densa, by the kidney’s baroreceptors, and by the negative feedback of ANG II (38). Thus the abolition of one of these factors would not necessarily result in decreased synthesis of this enzyme. Our Western blot analysis showed no differences in renin expression in the
studied groups, but it is possible that anesthesia has influenced the results, as shown by Hopf et al. (29). These results are in agreement with the findings of Golin et al. (25). These authors studied the effect of denervation on the expression of renin mRNA in the kidney and the renin secretion in different experimental conditions (by varying the content of sodium in the diet) and observed that in animals with normal diet, denervation did not change the renin mRNA, suggesting that under these conditions the synthesis of renin can occur independently of nerve activity. Draper et al. (19) also assessed renal renin content and its secretion after renal denervation in mature ovine fetus sheep. These authors also found that renal denervation did not alter the content of renin in the kidneys, but caused reduction of renin release. In fact, the data concerning renin secretion after denervation are conflicting. Säynävälammi et al. (54) and Winternitz et al. (68) did not find variations in plasma renin activity following denervation of spontaneously hypertensive rat; however, other studies showed reduction in plasma renin or prorenin after surgical renal denervation (37, 64).

In some models of programmed hypertension, including intrauterine growth restriction, levels of circulating catecholamines are increased, suggesting participation of the sympathetic nervous system in the genesis of hypertension (14, 28, 48). Rats exposed in utero to dexamethasone developed hypertension (14). These animals presented increased renal norepinephrine content and increased expression of sodium transporters in the kidney, suggesting the involvement of sympathetic system in the development of hypertension. Moreover, renal denervation reduced blood pressure in these rats (14). Therefore it seems...
that increased renal nerve activity contributes to hypertension induced both by in utero exposure to glucocorticoids, and to in utero exposure to high levels of glucose.

During diabetic pregnancy, high levels of glucose reach the fetus and stimulate it to produce insulin (13). Studies in experimental animals and in humans have shown that hyperinsulinemia may cause sympathetic overactivation due to its effects on the central nervous system (55). Fetal leptin levels also appear increased by maternal diabetes (47, 60), which may contribute to the central leptin resistance (60) and hypothalamic dysfunction (49) seen in offspring from hyperglycemic rats. In addition, leptin can act on the central nervous system to cause cardiovascualr baroreceptor reflex dysfunction (6). Thus, it is possible that hormonal changes that occurred during fetal development in our model caused central disarrangements that persisted in some degree during adult life.

The enhanced basal renal nerve activity observed in the offspring from diabetic mothers may also be related to alterations in the renin-angiotensin-aldosterone system. The intrarenal renin-angiotensin system studied in mice at birth was increased in the offspring of diabetic mothers (63). In addition, angiotensin I-converting enzyme activity was increased in heart, kidney, liver, and lung (67) from offspring of diabetic mothers. Magaton et al. (40) observed no increase in renal ANG II content in young offspring from diabetic dams, but levels of ANG 1–7 were reduced (40). Several studies have shown that ANG 1–7 and ANG II have opposite effects on vascular resistance and proliferation (35) and have pointed to ANG 1–7 as a modulator of the action of ANG II (53). Impaired baroreflex sensitivity in betamethasone-induced fetal programming may be due to progressive loss of ANG 1–7 function and an imbalance of ANG 1–7 and ANG II (57).

ANG is known to act on the central nervous system to stimulate renal sympathetic nerve activity and blunt the arterial baroreflex (16). This effect of ANG may involve the paraventricular nucleus of the hypothalamus (PVN), an important site for sympathetic integration and blood flow regulation (15, 27), and a region known to be altered in offspring of diabetic mothers (49). ANG II acts in the PVN to cause a sustained increase in sympathetic activity (15), which in turn stimulates the release of renin, closing a sequence of events that perpetuates angiotensinergic and sympathetic activation. The central effect of ANG II seems to be mediated by increased production of superoxide anions in the PVN and in the rostroventrolateral medulla (10). It is known that oxidative stress is increased in utero in offspring of diabetic mothers due to the depletion of antioxidants by hyperglycemia and the generation of reactive oxygen species. Oxidative stress was also increased after birth in offspring of diabetic mothers (50). Oxidative stress promotes generation of superoxide, and this can stimulate sympathetically neural activity, including to the kidney. Reactive oxygen species can increase blood pressure directly by acting on the sympathetic nerves or ganglia at the neuroeffector junction and indirectly by scavenging nitric oxide and reducing its bioavailability (32).

Whether the effect of renal denervation depends on afferent or efferent nerves is not clear, as both were cut in the present study. Our results suggest that hypertension and renal dysfunction in OD are caused by increased rRNA, but we cannot exclude a role of renal sensory afferent nerves and a loss of renorenal inhibitory reflexes in the genesis of hypertension and renal dysfunction.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


