Renal interstitial hydrostatic pressure and sodium excretion during acute volume expansion in diabetic rats

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Patel, Kaushik P., and Pamela K. Carmines. Renal interstitial hydrostatic pressure and sodium excretion during acute volume expansion in diabetic rats. Am J Physiol Regulatory Integrative Comp Physiol 281: R239–R245, 2001.—Experiments were performed to test the hypothesis that the renal interstitial hydrostatic pressure (RIHP) response to acute volume expansion is suppressed in diabetes mellitus. Sprague-Dawley rats received streptozotocin (STZ rats; 65 mg/kg ip) or vehicle (Sham rats). Two weeks later, RIHP and Na⁺ excretion responses to acute graded volume expansion with isotonic saline were quantified under Inactin anesthesia (0.1 mg/kg ip). In Sham rats, acute graded volume expansion to 10% body wt produced increases in RIHP (Δ = 12.2 ± 2.4 mmHg), urine flow (Δ = 54 ± 8 μl·min⁻¹·g⁻¹), and Na⁺ excretion (Δ = 11.5 ± 1.9 μeq·min⁻¹·g⁻¹) in STZ rats, these volume expansion-induced responses were significantly blunted (RIHP by 50%, urine flow by 81%, and Na⁺ excretion by 76%). Renal denervation normalized the RIHP response to volume expansion and improved the diuretic and natriuretic responses in STZ rats. Moreover, diuretic and natriuretic responses to direct changes in RIHP (induced by renal interstitial volume expansion) were blunted in STZ rats. We conclude that diminished alterations in RIHP, as well as a reduced impact of RIHP on Na⁺ excretion, contribute to the impaired diuretic and natriuretic responses to acute volume expansion during the early stage of diabetes.

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STZ (65 mg/kg ip; Sigma, St. Louis, MO) in a 2% solution of cold 0.1 M citrate buffer (pH 4.5). Sham rats received a similar volume of vehicle. Onset of diabetes occurred rapidly in STZ rats and was identified by polydipsia, polyuria, and blood glucose concentration >250 mg/dl (Accu-chek, Boehringer Mannheim, Indianapolis, IN).

**Surgical Procedures**

Two weeks after injection with STZ or vehicle, rats were anesthetized with Inactin (100 mg/kg ip). Body temperature was maintained between 36° and 38°C via external warming. Tracheal intubation facilitated spontaneous respiration. The left femoral artery was cannulated with PE-50 polyethylene tubing and connected to a pressure transducer (Gould P23 ID) for the continuous recording of arterial pressure. The left femoral vein was cannulated with PE-50 tubing for infusion of isotonic saline. A 0.7-ml bolus of isotonic saline containing 40 mg/ml inulin was administered via a cannula in the left femoral vein, followed by a sustaining infusion containing inulin (20 mg/ml) at a rate of 20 μl/min. The left kidney was exposed through a midline incision. Placement of a PE-10 cannula in the left ureter allowed urine collection into pre-weighed tubes. The measurement of RIHP was performed via techniques previously validated by García-Estan and Roman (8). Briefly, the interstitial catheters were constructed from polyethylene matrix material with 60-μm pores that are impermeable to solid tissue yet allow free communication with the interstitial fluid compartment. Each polyethylene matrix was cut into a cylinder 1.0 mm in diameter and 2.0 mm in length. The polyethylene matrix was inserted into PE-50 tubing and tied with 4–0 silk for permanent attachment. An 18-gauge needle was used to make a small nick in the ventral side of the left kidney to a depth of 3–4 mm. A cautery was used to stop the bleeding, and the polyethylene matrix attached to PE-50 tubing was advanced into the renal parenchyma. The catheter was fixed in its position with quick glue. Probe viability was confirmed by observation of an increase in RIHP during brief compression of the renal vein with a cotton swab, as documented previously (8).

**Experiment Protocols**

**Experiment 1: effect of IDDM on RIHP and excretory responses to acute volume expansion.** After a 30-min equilibration period, two baseline urine collections (10 min each) were obtained. Subsequently, acute intravenous infusion of isotonic saline was employed to achieve 10% volume expansion over a 40-min period (0.25% body wt/min; e.g., for a 250-g rat, saline was infused at a rate of 0.625 ml/min for 40 min). Urine was collected at the 5-, 10-, 15-, 20-, 30-, and 40-min time points during imposition of the volume expansion. These collection intervals are coincident with cumulative volume expansion to 10%, 20%, 30%, 40%, 50%, and 100% body wt, respectively.

**Experiment 2: effect of renal decapsulation on responses to volume expansion in Sham and STZ rats.** These experiments were performed to test the hypothesis that the diminished ability of diabetic rats to excrete an acute volume load reflects suppressed alterations in RIHP. Sham and STZ rats were prepared as described above, except that the left kidney of each was decapsulated. After obtaining baseline measurements, excretory and RIHP responses to acute volume expansion to 10% body wt in 40 min were performed. Renal decapsulation is known to prevent the changes in RIHP that accompany acute volume expansion (25). Using this method, we could eliminate the differences in RIHP between the two groups and therefore watch for the difference in excretory responses unrelated to changes in RIHP.

**Experiment 3: effect of acute renal denervation on excretory and RIHP responses to volume expansion in Sham and STZ rats.** We previously demonstrated that renal denervation either improved or corrected the blunted excretory response to acute volume expansion in diabetic rats (20, 24, 30). To determine if the beneficial impact of denervation involves improvement of the RIHP response to volume expansion, Sham and STZ rats were anesthetized and surgically prepared for urine collection and RIHP measurement. The renal nerves were cut bilaterally, and, after a 30-min equilibration period, the volume expansion protocol was completed as described for experiment 1.

**Experiment 4: effect of direct renal interstitial volume expansion on RIHP and excretory function in Sham and STZ rats.** These experiments examined the hypothesis that diabetes suppresses the excretory response to changes in RIHP. Anesthetized Sham and STZ rats were prepared for urine collection and RIHP monitoring. After obtaining baseline measurements, acute direct renal interstitial volume expansion (DRIVE) was achieved by injecting 100 μl of 2% albumin in saline solution into the renal interstitium via an additional adjacent intrarenal catheter.

**Analyses**

Urine volume was measured gravimetrically. The Na+ concentration of each urine sample was measured using an ion-selective electrode (Beckman ion analyzer, Irvine, CA). Inulin concentrations were determined using the anthrone method (4, 27). Whole kidney glomerular filtration rate (GFR) was calculated as the clearance of inulin. Statistical analysis employed repeated-measures ANOVA followed by multiple-range test for comparison of means among groups (29). GFR and fractional excretion of Na⁺ (FENa) values were calculated for the initial control period and the 10% volume expansion period, and these values were compared between the groups using a t-test. P values <0.05 were considered to indicate statistical significance.

**RESULTS**

**Animal Characteristics**

Table 1 details the salient characteristics of Sham and STZ rats used in this study. Blood glucose levels were significantly elevated in STZ rats (360 ± 19 mg/dl; n = 36) relative to Sham rats (92 ± 11 mg/dl; n = 31). Despite having similar body weights on the date of STZ or vehicle injection, STZ rats exhibited little weight gain over the ensuing 2-wk period. Accordingly, Sham rats were significantly larger than STZ rats at the time of the terminal experiment, although renal hypertrophy was evident in STZ rats. Bradycardia was evident in STZ rats; however, mean arterial pressure did not differ between Sham and STZ rats under baseline conditions, and acute volume expansion did not significantly alter this parameter in either group (data not shown).

**Experiment 1: effect of IDDM on RIHP and excretory responses to acute volume expansion.** Urine flow and sodium excretion responses to acute volume expansion from intact left kidneys of Sham and STZ rats are shown in Fig. 1, A and B, respectively. As we previously reported for studies performed 2 or 4 wk after...
STZ treatment (20, 30), both the diuretic and natriuretic response to volume expansion were blunted significantly in the STZ rats relative to the Sham group. RIHP responses to acute volume expansion are shown in Fig. 1C. RIHP was significantly lower in STZ rats under baseline conditions (before volume expansion) and at all points during the volume expansion protocol. This phenomenon was not solely a reflection of the lower baseline RIHP value in STZ rats, as the volume expansion-induced rise in RIHP (ΔRIHP) was also blunted significantly in the STZ rats (Fig. 2). Time control experiments confirmed that no significant change in RIHP occurred in rats monitored for 40 min without volume expansion (data not shown). There were no significant differences in GFR or FENa before or at the end of acute volume expansion (10% volume expansion period) between Sham and STZ rats (Table 2).

Experiment 2: effect of renal decapsulation on responses to volume expansion in Sham and STZ rats. The effects of acute volume expansion on urine flow and sodium excretion responses after renal decapsulation are summarized in Fig. 3, A and B, respectively. In Sham rats with decapsulated kidneys, diuretic and natriuretic responses are substantially smaller in magnitude compared with intact (Fig. 1). However, there were no significant differences in diuresis and natriuresis between the Sham and STZ groups with renal decapsulation. In addition, there was no significant difference between the Sham and STZ groups with regard to the RIHP response to acute volume expansion after renal decapsulation (Fig. 3C). There were no significant differences in GFR or FENa before or at the end of acute volume expansion (10% volume expansion period) between Sham and STZ rats (Table 2).

Experiment 3: effect of acute renal denervation on excretory and RIHP responses to volume expansion in Sham and STZ rats. Sham and STZ rats subjected to acute renal denervation displayed similar RIHP values under baseline conditions and during acute volume expansion (Fig. 4C). The diuretic and natriuretic responses of STZ rats to acute volume expansion were markedly improved in the renal denervation group (Fig. 4, A and B), as was reported previously (20).

Table 1. Characteristics of Sham and STZ rats

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sham (n = 10)</th>
<th>STZ (n = 10)</th>
<th>Sham (n = 8)</th>
<th>STZ (n = 10)</th>
<th>Sham (n = 7)</th>
<th>STZ (n = 10)</th>
<th>Sham (n = 6)</th>
<th>STZ (n = 10)</th>
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<tbody>
<tr>
<td>Body Weight, g</td>
<td>298 ± 9</td>
<td>247 ± 9*</td>
<td>273 ± 16</td>
<td>232 ± 9*</td>
<td>300 ± 6</td>
<td>238 ± 10*</td>
<td>315 ± 21</td>
<td>209 ± 15*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>119 ± 3</td>
<td>116 ± 7</td>
<td>97 ± 5</td>
<td>102 ± 5</td>
<td>96 ± 8</td>
<td>112 ± 9</td>
<td>124 ± 4</td>
<td>115 ± 5</td>
</tr>
<tr>
<td>Blood [Glucose], mg/dl</td>
<td>97 ± 14</td>
<td>343 ± 26*</td>
<td>89 ± 5*</td>
<td>349 ± 17*</td>
<td>78 ± 12</td>
<td>332 ± 21*</td>
<td>103 ± 12</td>
<td>416 ± 11*</td>
</tr>
<tr>
<td>Heart Rate, beats/min</td>
<td>380 ± 16</td>
<td>293 ± 17*</td>
<td>406 ± 14</td>
<td>303 ± 10*</td>
<td>396 ± 14</td>
<td>310 ± 15*</td>
<td>350 ± 22</td>
<td>308 ± 9*</td>
</tr>
<tr>
<td>Left Kidney Weight, g</td>
<td>1.02 ± 0.06</td>
<td>1.27 ± 0.06*</td>
<td>0.94 ± 0.06</td>
<td>1.18 ± 0.06*</td>
<td>0.95 ± 0.06</td>
<td>1.20 ± 0.04*</td>
<td>1.07 ± 0.08</td>
<td>1.21 ± 0.11*</td>
</tr>
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</table>

Values represent means ± SE. Sham rats, rats receiving vehicle; STZ rats, rats receiving streptozotocin; MAP, mean arterial pressure; [Glucose], glucose concentration. *P < 0.05 vs. Sham.

Fig. 1. A: urine flow (μl·min⁻¹·g kidney wt⁻¹), B: sodium excretion (μeq·min⁻¹·g·kidney wt⁻¹), and C: renal interstitial hydrostatic pressure (RIHP, mmHg) responses to acute volume expansion (% body wt) with isotonic saline from intact kidneys in rats receiving vehicle (Sham rats) and those receiving streptozotocin (STZ rats). Values represent means for each parameter ± SE. *P < 0.05 vs. Sham.
However, despite complete normalization of the RIHP response, the Na\textsuperscript{+} excretory response to acute volume expansion was not fully restored by renal denervation in STZ rats. There were no significant differences in GFR or FENa before or at the end of acute volume expansion (10% volume expansion period) between Sham and STZ rats (Table 2).

Experiment 4: effect of DRIVE on RIHP and excretory function in Sham and STZ rats. Figure 5C depicts the impact of IDDM on the RIHP response to DRIVE (100 \textmu l of 2% albumin in saline solution). DRIVE evoked similar increments in RIHP in both groups of rats; however, the urine flow and sodium excretion responses to this maneuver were significantly blunted in STZ rats (Fig. 5, A and B).

Table 2. Glomerular filtration rate and fractional excretion of sodium in Sham and STZ rats before and at the last period of volume expansion

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sham (n=10)</th>
<th>STZ (n=10)</th>
<th>Sham (n=8)</th>
<th>STZ (n=10)</th>
<th>Sham (n=7)</th>
<th>STZ (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR, ml/min\textsuperscript{-1}g kidney wt\textsuperscript{-1}</td>
<td>0.82±0.11</td>
<td>1.85±0.12</td>
<td>0.22±0.12</td>
<td>4.30±1.21</td>
<td>1.13±0.11</td>
<td>1.81±0.12</td>
</tr>
<tr>
<td>FENa, %</td>
<td>0.97±0.10</td>
<td>2.36±0.09</td>
<td>0.22±0.11</td>
<td>11.88±3.65</td>
<td></td>
<td></td>
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DISCUSSION

The results of the present study reveal a diminished increase in RIHP concomitant with a blunted natriuresis and diuresis in response to acute volume expansion in STZ-induced diabetes mellitus. Renal decapsulation eliminated the disparities in the RIHP and excretory responses to acute volume expansion, primarily by suppressing responses in Sham rats. Although renal denervation normalized the RIHP responses to acute volume expansion in STZ rats, it did not fully restore the Na\textsuperscript{+} excretory response. Finally, DRIVE-induced increases in RIHP were less effective in eliciting diuresis and natriuresis in STZ rats than in Sham rats. These observations suggest that the blunted renal excretory response to acute volume expansion in IDDM involves both a diminished alteration in RIHP (due largely to the functional impact of renal nerves) and a reduced excretory responsiveness to changes in RIHP.
Altered fluid balance, blood volume, and exchangeable Na\(^+\) characterize diabetes mellitus. Studies of exchangeable Na\(^+\) in subjects with diabetes of short duration without complications indicate that Na\(^+\) retention early in the disease may be relatively common (5, 17, 28). A 10–15% increase in exchangeable Na\(^+\) content is evident in metabolically stable diabetic subjects (normotensive or hypertensive) as well as those with or without complications (5, 17, 28). Compared with normal individuals, the natriuretic response to volume expansion induced by water immersion is reduced in diabetic patients (15, 16, 18). Furthermore, diabetics in metabolic control excrete 50% less Na\(^+\) than normal individuals after an acute intravenous Na\(^+\) load (19). Consistent with these observations, we have demonstrated that the Na\(^+\)-excretion response to acute saline loading (the so-called volume reflex) is reduced in rats studied 2 or 4 wk after the induction of diabetes by STZ injection (20, 30).

The mechanisms responsible for the blunted excretory response to volume expansion observed in diabetic rats could be either extrinsic or intrinsic to the kidney or both. Previous work from our laboratory revealed that renal denervation improves the volume reflex in diabetic rats (20) and that the renal sympathoinhibition response to acute volume expansion is attenuated in diabetic rats (24). In addition, the natriuretic response to exogenously infused atrial natriuretic factor (ANF) is attenuated in diabetic rats (21), whereas basal ANF levels are higher in diabetics and do not increase appropriately during Na\(^+\) loading (11, 19). Although these data indicate that renal nerves and ANF may be partly responsible for the blunted volume reflex in diabetics, they do not fully account for the blunted response to volume expansion. Thus altered intrarenal mechanisms controlling natriuretic response to acute volume expansion may play a significant role in the blunted natriuretic response to this maneuver.

It is well known that increases in RIHP represent an important factor that reduces Na\(^+\) reabsorption by the renal tubule. Increases in RIHP are a known consequence of volume expansion and are thought to play a role in the blunted natriuretic response to volume expansion in diabetics.

**Fig. 4.** A: urine flow (μl·min\(^{-1}\)·g kidney wt\(^{-1}\)), B: sodium excretion (μeq·min\(^{-1}\)·g kidney wt\(^{-1}\)), and C: RIHP (mmHg) responses to acute volume expansion (% body wt) with isotonic saline from denervated kidneys in Sham and STZ groups. Values represent means for each parameter ± SE. *P < 0.05 vs. Sham.

**Fig. 5.** A: urine flow (μl·min\(^{-1}\)·g kidney wt\(^{-1}\)), B: sodium excretion (μeq·min\(^{-1}\)·g kidney wt\(^{-1}\)), and C: ΔRIHP (mmHg) responses to direct renal interstitial volume expansion (DRIVE; 100 μl of 2% albumin in saline solution) in Sham and STZ rats. Values represent means for each parameter ± SE. *P < 0.05 vs. Sham.
proximal tubule and loop of Henle during volume expansion (9). In the present study, basal RIHP levels were lower in STZ rats than in Sham rats. Moreover, at each level of acute volume expansion, RIHP was significantly lower in the STZ rats compared with Sham rats, concomitant with a lower urine flow and Na⁺ excretion. This phenomenon cannot be explained purely by the initial differences in RIHP between the groups, as the absolute change in RIHP was also suppressed in STZ rats (Fig. 2). The lower RIHP and its diminished response to acute volume expansion can be expected to increase proximal Na⁺ reabsorption during volume loading in STZ rats, resulting in a blunted natriuretic response. When the differences in the RIHP response to volume expansion between STZ and Sham rats were abolished by renal decapsulation, between-group disparities in the diuretic and natriuretic responses to acute volume expansion were also abolished (Fig. 3). This phenomenon was primarily the result of the ability of renal decapsulation to suppress RIHP and its responsiveness to acute volume expansion in normal rats (13). In fact, responses of STZ rats with intact kidneys to acute volume expansion were similar to those exhibited by decapsulated Sham kidneys. These data indicate that diminished RIHP responsiveness to volume loading may be an important factor in the volume reflex in STZ rats.

As previously reported from our laboratory, renal nerves contribute to the blunted diuretic and natriuretic response to acute volume expansion in diabetic rats. Because renal denervation improves the volume reflex in rats with diabetes (21, 30), it was postulated that renal denervation may exert its beneficial influence on the volume reflex by promoting the effect of acute volume expansion on RIHP. Indeed, renal denervation normalized both basal RIHP and the changes in RIHP evoked by acute volume expansion in STZ rats. These changes translated into improvement of the blunted excretory response to acute volume expansion in STZ rats. Based on these observations, it appears likely that the impact of renal sympathetic nerve activity to suppress the volume reflex in diabetes arises primarily via a decrease in RIHP and its responsiveness to volume expansion.

The mechanism through which changes in RIHP alter Na⁺ reabsorption appears to involve alterations in medullary blood flow and capillary pressure (3, 26). According to this proposal, acute volume expansion is associated with a shift in intrarenal blood flow to deep cortex and medulla. The increase in medullary blood flow leads to a change in vasa recta capillary pressure, an increase in RIHP, and dissipation of the medullary solute gradient. According to this hypothesis, an alteration in intrarenal blood flow distribution may modulate RIHP responses to acute volume expansion in diabetic rats, ultimately influencing urine flow and Na⁺ excretion. It is through such a mechanism that nitric oxide is proposed to promote the excretory and RIHP responses to acute volume expansion (1). The effect of diabetes mellitus on medullary blood flow responses to acute volume expansion remains to be examined.

It is important to note that normalization of volume expansion-induced increases in RIHP (by renal denervation) did not totally restore the excretory response to volume expansion in the STZ rats. These data suggest that for a given change in RIHP there is a reduced excretory response in STZ rats. To further address this postulate, we imposed DRIVE on kidneys from Sham and STZ rats. The fact that this maneuver provoked similar increments in RIHP in Sham and STZ rats indicates that the compliance of the interstitial compartment is similar in both groups. However, the urine flow and Na⁺ excretory responses to DRIVE (considered a direct means of increasing RIHP) were blunted in STZ rats compared with Sham rats. These data suggest an abnormality in the transduction of changes in RIHP into subsequent diuresis and natriuresis in STZ rats. Similar blunted excretory response to changes in RIHP is observed in pregnant rats (12). Interestingly, pregnant rats also have a blunted excretory response to acute volume expansion, which is also dependent on tonic renal sympathetic nerve activity (23). The converse situation is evident in spontaneously hypertensive rats, which respond to a step increment in RIHP with an exaggerated Na⁺-excretion response compared with Wistar-Kyoto rats (12). Accordingly, there is exaggerated excretory response to acute volume expansion in spontaneously hypertensive rats compared with Wistar-Kyoto rats (22). We suggest that an impaired transduction of changes in RIHP into diuresis and natriuresis may be responsible for the “denervation-resistant” component of the impaired volume reflex in diabetic rats, although further studies are required to fully explore this postulate.

In summary, the present study indicates that diminished alterations in RIHP due to presence of tonic renal sympathetic nerve activity and reduced excretory responses to given change in RIHP result in blunted renal excretory responses to acute volume expansion in IDDM.

**Perspectives**

Diabetic patients have been reported to have a reduced natriuretic response to volume expansion by water immersion (18). Hemodynamic or hormonal changes could not account for this altered sodium excretion in diabetic patients (15, 16). We recently demonstrated that removal of renal nerves attenuates the altered excretory responses to acute volume expansion in diabetic rats (20, 30). This study demonstrates for the first time that intrarenal events are responsible, at least in part, for the blunted renal excretory response to acute volume expansion in rats with diabetes. The pathophysiological mechanisms producing an abnormal response in RIHP to acute isotonic saline load, as well as the manner in which the abnormal RIHP response affects renal Na⁺ and water handling, remain to be elucidated.
REFERENCES


