Salt-resistant blood pressure and salt-sensitive renal autoregulation in chronic streptozotocin diabetes

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Lau C, Sudbury I, Thomson M, Howard PL, Magil AB, Cupples WA. Salt-resistant blood pressure and salt-sensitive renal autoregulation in chronic streptozotocin diabetes. Am J Physiol Regul Integr Comp Physiol 296: R1761–R1770, 2009. First published April 1, 2009; doi:10.1152/ajpregu.90731.2008.—Hyperfiltration occurs in early type 1 diabetes mellitus in both rats and humans. It results from afferent vasodilation and thus may impair stabilization of glomerular capillary pressure by autoregulation. It is inversely related to dietary salt intake, the “salt paradox.” Restoration of normal glomerular filtration rate (GFR) involves increased preglomerular resistance, probably mediated by tubuloglomerular feedback (TGF). To begin to test whether the salt paradox has pathogenic significance, we compared intact vs. diabetic (streptozotocin) Long-Evans rats with normal and increased salt intake, 1 and ~3% by weight of food eaten, respectively. Weekly 24-h blood pressure records were acquired by telemetry before and during diabetes. Blood glucose was maintained at ~20 mmol/l by insulin implants. GFR was significantly elevated only in diabetic rats on normal salt intake, confirming diabetic hyperfiltration and the salt paradox. Renal blood flow dynamics show strong contributions to autoregulation by both TGF and the myogenic mechanism and were not impaired by diabetes or by increased salt intake. Separately, systolic pressure was not elevated in diabetic rats at any time during 12 wk with normal or high salt intake. Autoregulation was effective in all groups, and the diabetic-normal salt group showed significantly improved autoregulation at low perfusion pressures. Histological examination revealed very minor glomerulosclerosis and modest mesangial expansion, although neither was diagnostic of diabetes. Periodic acid-Schiff-positive droplets found in distal tubules and collecting duct segments were diagnostic of diabetic kidneys. Biologically significant effects attributable to increased salt intake were abrogation of hyperfiltration and of the left shift in autoregulation in diabetic rats.

DIABETES MELLITUS AND HYPERTENSION are the two of the most important causes of glomerular disease, leading ultimately to renal replacement therapy. The hypertensive injury is hemo-dynamically (physically) mediated or initiated (23, 45). The causes of diabetic glomerular injury appear to be more complex, as there is a great deal of evidence for dysfunctional cell signaling (12, 21). However, there is clear evidence that hypertension is involved in diabetic nephropathy (6), and, in fact, hypertension accelerates glomerular injury due to any other etiology (23). These observations highlight the importance of both the primary insult (here blood pressure or diabetes) and the susceptibility of the target organ to injury. Renal susceptibility appears to be relatively generic and independent of the specific insult (22, 62).

Here, we examine the possibility that type 1 diabetes mellitus increases susceptibility to hypertension-induced, or hypertension-associated, renal disease in ways that can be modified. A strong candidate mechanism is diabetic hyperfiltration, which arises from dysregulation of proximal reabsorption so that at any glomerular filtration rate (GFR), a disproportionately dilute fluid reaches the macula densa (84). The resulting reduction of preglomerular resistance is mediated by tubuloglomerular feedback (TGF). Diabetic rats and humans both exhibit GFR that is inversely proportional to salt intake. This “salt paradox” was first described, in both species, as an increase in GFR, resulting from reduced salt intake (54, 88). The original observation was then extended to show that modestly increased dietary salt intake abrogates diabetic hyperfiltration in rats (85). In the absence of any other effects of elevated salt intake, a relative increase of pregglomerular resistance would predict reduced renal susceptibility to pressure-induced or pressure-associated injury (1).

Autoregulation, mediated by the myogenic mechanism and TGF, is the only control system that stabilizes glomerular capillary pressure when blood pressure fluctuates or increases. Impaired autoregulation would also be predicted to increase renal susceptibility (26, 46). In addition to decreasing preglomerular resistance, diabetes is often considered to impair autoregulation, although the literature is rather mixed (13, 16, 28, 30, 36, 50, 51, 56, 78). In early diabetes (type 1 and type 2), there is increased neuronal nitric oxide synthase (nNOS) protein at the macula densa (76, 93). Typically, nitric oxide generated by this nNOS limits the dynamic range, or regulatory efficiency, of TGF (75). Under normal conditions, macula densa nNOS is regulated by TGF, whereas in early diabetes, it is to some extent released from this control (76). From the reduced ability of TGF to regulate glomerular capillary pressure and from the role of TGF in autoregulation (15), one can predict reduced TGF-myogenic interaction (40, 71) with potential impairment of autoregulation. Thus, it is reasonable to consider the dysregulation of preglomerular resistance that occurs in the salt paradox as a modifiable susceptibility factor.

To date, there has been no systematic evaluation of long-term implications of the salt paradox. There are two components to this problem. While mitigation of diabetic hyperfiltration by increased preglomerular resistance is predicted to reduce renal susceptibility to hypertension induced or associated injury, any such mitigation would, of course, be negated if the treatment increased blood pressure or if it impaired auto-
regulation (for instance if angiotensin-dependent modulation of TGF and/or the myogenic mechanism were blunted). In this study, we address the second question: whether an increase of salt intake that is sufficient to abrogate the diabetic hyperfiltration affects blood pressure and renal autoregulation.

METHODS

All procedures involving animals were approved by the University of Victoria Animal Care Committee and were consistent with the Guidelines promulgated by the Canadian Council on Animal Care. Male Long-Evans rats were acquired from Charles River (Montreal, Quebec, Canada). Rats had free access to food and fluid at all times, except as noted specifically below. Unless specifically noted, rats were fed Purina LabDiet 5001 containing 0.4% sodium and 0.67% chloride by weight. Surgical preparations for the experiments were initiated when the rats were ~10 wk of age, except in experiment 2 where initiation occurred at ~21 wk of age so that rats were age matched with experiment 3.

Under sterile conditions and isoflurane anesthesia with analgesia (buprenorphine, 0.01 mg/kg ip, Temgesic; Reckitt & Benckiser, Mississauga, Ontario, Canada), a pressure telemeter (model PA11-C40; Data Sciences International, St. Paul, MN) having a 10-cm cannula was implanted subcutaneously in the flank and secured by a purse string suture. The cannula tip was inserted in the femoral artery and advanced into the aorta to the level of the renal arteries, leaving slack to accommodate growth. After 12–14 days, when normal circadian rhythms were restored, a control 24-h record was acquired in every rat. Then diabetes was induced in some rats by intraperitoneal injection of freshly prepared streptozotocin (50 mg/kg in ice-cold saline) in isoflurane-anesthetized rats. Streptozotocin injections were repeated if necessary. Blood glucose was measured biweekly in all animals (Ascensia glucometer); blood was acquired from the tail of conscious, unrestrained rats. When diabetes was confirmed by blood glucose, rats received insulin by subcutaneous implantation of one-half a LinPlant (Linshin, Canada, Toronto, Ontario, Canada) to maintain blood glucose at ~20 mmol/l. LinPlants were renewed at ~4-wk intervals throughout the course of the experiments.

Experiments. Three experiments were performed in which blood pressure was measured by telemetry on a weekly basis, while body weight and blood glucose were measured twice weekly.

The first experiment used 12 rats, half of which were diabetic. Beginning 2 wk after induction of diabetes, three 24-h records of blood pressure were acquired at 7-day intervals with salt intake first reduced to 0.26% (AIN-93M, Dyets, Bethlehem, PA), then restored to 1%, then increased to 4%. A further doubling of salt intake was attempted, but proved aversive and therefore uninformative. Data were acquired 5–7 days after changing salt intake. Approximately a week after the last pressure recording and on normal salt intake, steady-state autoregulation was assessed under anesthesia.

The second experiment employed a published protocol (86) to demonstrate the salt paradox in our rats and to investigate renal hemodynamics. Four groups of rats were used [Intact normal and high salt, n = 5 each (IN-N and IN-H), and diabetic normal and high salt, n = 7 each (DM-N and DM-H)]. Diabetes was induced after the control record was acquired, and 3 wk later, salt intake was elevated to 2.5% by weight of food intake in half of the rats. Renal hemodynamics and GFR were measured after the 4th week. Renal blood flow (RBF) dynamics were assessed before and after intravenous injection of 10 mg/kg of L-NAME to block nitric oxide production.

In the third experiment, diabetes was induced after the control record was acquired. Salt intake was increased at week 3 and continued until week 13 to evaluate the evolution of cardiovascular variables. For workload reasons, it was performed in two iterations, each containing the same four groups as in the previous experiment. The first iteration had n = 4 in each group, while the second iteration had n = 5 for both IN-N and IN-H and n = 7 for both DM-N and DM-H. Increasing salt intake from 1% to 2.5% in the first leg had little or no discernable effect. Thus, in the second leg, salt intake was increased to 3.5% of food intake by weight. Results from the two legs were not different and were pooled for presentation. At the end of the protocol, renal autoregulation was assessed by standard methods, and the left kidney of each rat was harvested for histological analysis.

Data acquisition and analysis. Telemetry data were acquired from every rat in a 24-h record once per week. Data were acquired at 250 Hz in 10-s bursts every 2 min throughout the day. Heart rate and systolic pressure are reported; we chose to report systolic rather than mean pressure because analysis showed that mean and diastolic pressures contain the same information as systolic pressure. It also facilitates comparison with a previous long-term study of diabetic rats that reported systolic pressure (6). Because there are reports in the literature that altered salt intake can change circadian rhythms of cardiovascular variables, records were inspected to assure that these rhythms were normally aligned on dark-light boundaries. Dark-light differences in heart rate and systolic pressure were quantified in selected records.

Renal hemodynamics were assessed by standard protocols (71, 90, 92). Briefly, rats received buprenorphine (0.02 mg/kg) and were anesthetized by isoflurane. Cannulas were placed in the trachea to facilitate ventilation, a femoral artery for measurement of renal perfusion pressure, and a femoral vein for infusions. Femoral arterial pressure was measured by a Kent pressure transducer (TRN050) driven by a TRN005 amplifier. Renal blood flow was measured by a Transonic Systems model T401 transit time ultrasound flowmeter (1PRB probe on the renal artery). A motorized occluder was placed on the aorta between the right and left renal arteries and was used to force blood pressure (71, 89). GFR was measured by the clearance from the left kidney of FITC-inulin (Sigma) over intervals of 25–30 min with fluorescence measured by a Perkin-Elmer Victor 5 fluorimeter (48). At the end of the hemodynamics procedures, in experiment 3 only, the left kidney was removed, split and blotted, and then fixed by immersion in 10% neutral buffered formalin. After embedding in paraffin, 4-µm sagittal sections were cut and stained with periodic acid Schiff (PAS) and counterstained with hematoxylin to show nuclei. The slides were examined by a renal pathologist (A. B. Magil) in a single blind fashion. All glomeruli in a section were examined.

Blood pressure and RBF signals from terminal experiments were filtered at 40 Hz and sampled at 200 Hz online. Data segments of 1311 s (217 points) were low pass filtered at 1 Hz using a fast Fourier transform (FFT)-inverse FFT filter with a rectangular window and subsampled to 3.125 Hz. Power spectra, transfer functions, and coherences based on the FFT were computed by standard algorithms (71) using 1024 point segments subjected to linear trend removal and shaped by the Hann window. The slope of gain reduction by a control system and the magnitude of the associated phase peak provide information about the internal dynamics of that system. A system that responds only to the level of the input variable, will typically have a slope of gain reduction ~20 dB/decade and a phase peak of π/4 rad at the corner frequency, whereas a system that responds also to the rate of change of the input variable will have slope ~40 dB/decade and a phase peak of π rad (41, 53).

Statistical analysis was performed using routines included in Statistica v5.5 (Statsoft, Tulsa, OK). Initial testing of telemetry data involved 2-way, repeated-measures ANOVA, with planned contrast analysis to assess responses to diabetes, to increased dietary salt, and time-dependent effects. Some potential effects of diabetes and altered salt intake were obscured by differences in the initial control periods. Therefore, evolution of systolic pressure was also assessed by least squares fitting of the weekly data and testing of slopes by 2-way ANOVA. The effects of diabetes and salt intake upon renal hemodynamic and clearance data were tested by 2-way ANOVA, completed by planned contrast analysis. Data are presented as means ± SE, and P ≤ 0.05 is considered to indicate a significant difference.
RESULTS

Experiment 1: chronic renal function curve and steady-state autoregulation. Blood glucose, averaged over the 3 wk of the chronic renal function curve, was 4.9 ± 0.2 mmol/l in the intact animals and was 18.1 ± 2.2 mmol/l (P < 0.001) in the diabetic rats. Figure 1 shows the chronic renal function curves obtained from intact and diabetic rats. In both groups, 24-h average systolic pressure was independent of salt intake over this 15-fold range. As shown in Fig. 2, both intact and diabetic rats displayed effective autoregulation when renal perfusion pressure was greater than 90 mmHg. The diabetic rats maintained constant RBF to lower pressures so that normalized RBF at 80 and 90 mmHg was greater in the diabetic than in the intact rats (P = 0.05 and P = 0.025, respectively).

Experiment 2: diabetic hyperfiltration, the salt paradox, and RBF dynamics. RBF, GFR, and filtration fraction were assessed after 4 wk of poorly controlled diabetes with high salt intake in the last week. RBF was not affected by diabetes, by salt intake, or by interaction between the two (Table 1). In contrast, GFR was increased by diabetes, and this effect was evident only in diabetic rats on normal salt intake. Consequently, filtration fraction was elevated in DM-N rats but not in DM-H.

Blood glucose remained low in the intact rats (IN-N: 4.6 ± 0.3 mmol/l; IN-H 4.4 ± 0.2 mmol/l) but was elevated to 20.9 ± 1.3 in DM-N and 21.8 ± 1.9 mmol/l in DM-H. Adding salt to the diet in the 4th week had no effect on blood glucose in diabetic or intact rats. Intact rats gained 123 ± 8 g, while diabetic rats lost 50 ± 11 g; diabetic rats had slightly shorter snout-to-anus length and lower body weight-to-length ratio (Table 1). The latter indicates that body composition differed significantly (72), so data were not normalized to body weight. Neither left nor right kidney weight differed between intact and diabetic rats (Table 1). Nor was there any interaction between diabetes and salt intake on body length, weight-to-length ratio, or kidney weight.

Heart rate is shown in Fig. 3A and declined at the onset of diabetes, whereas minimal time-dependent reduction of heart rate was seen in IN-N and IN-H. There were main effects of diabetes and time on heart rate, and an interaction between them (all P < 10^-6), but no main or interactive effect of altered salt intake. Systolic pressure is shown in Fig. 3B and was stable in IN-N and IN-H groups (−0.1 ± 0.4 and −0.5 ± 0.2 mmHg/wk), but decreased upon induction of diabetes (−3.4 ± 0.4 and −2.0 ± 0.5 mmHg/wk in DM-N and DM-H, respectively, P = 10^-5 vs. intact). Although reduction of systolic pressure was greater in DM-N than in DM-H (P = 0.038), it occurred before salt intake was altered, indicating that it reflects natural variation and not a response to added dietary salt. The circadian rhythm in systolic pressure, shown by dark-light difference (Fig. 3C), showed a main effect of time (P = 0.0116) and no significant interaction terms. This resulted from an increased dark-light difference in DM-H between control and week 1 (P = 0.029), and in IN-N between weeks 1 and 2 (P = 0.0048). The former is probably, and the latter is certainly, unrelated to experimental challenges.

RBF dynamics were assessed under control conditions and after l-NAME. The coherence and transfer functions are presented in Fig. 4. All groups display the characteristic signatures of the myogenic mechanism and TGF, gain reductions below ~0.15 Hz and below 0.04 Hz, respectively, with their associated phase peaks. The slopes of gain reduction by the myogenic mechanism and amplitude of phase peaks are presented in Table 1. In the control period, ANOVA revealed that slope of gain reduction by the myogenic mechanism and TGF, gain reductions below 0.15 Hz and below 0.04 Hz, respectively, was less in IN-N (P < 0.05) and in IN-H and DM-N, while phase in IN-N was less than in DM-N. As expected, RBF was lower after l-NAME in all rats (Table 1). No modulation of RBF dynamics by l-NAME was visible in these groups. Only two records were obtained after l-NAME in the IN-N group. In both rats, the slope of gain reduction and the amplitude of the phase peaks increased after l-NAME.

Experiment 3: long-term consequences of increased dietary salt. Evolution of body weight and blood glucose in the long-term experiment are shown in Fig. 5. A and B, respectively. In both IN-N and IN-H, blood glucose was low and stable throughout the experiment. In DM-N and DM-H, blood glucose was close to the 20 mmol/l target. Body weights increased progressively in the intact rats. The diabetic rats showed a very small, albeit continuous, rise of body weight. Body length was only modestly less in diabetic than intact rats, shown in Table 2. Body weight-to-length ratio was markedly reduced (Table 2). Kidney weights are shown in Table 2 and were greater in diabetic than intact rats.

Heart rate declined rapidly upon induction of diabetes, shown in Fig. 5C. There were main effects of diabetes and of
Table 1. Hemodynamic assessment after 4 weeks of poorly controlled diabetes with high salt intake in the last week

<table>
<thead>
<tr>
<th></th>
<th>IN-N</th>
<th>IN-H</th>
<th>DM-N</th>
<th>DM-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length, cm</td>
<td>27.5±0.3</td>
<td>27.2±0.5</td>
<td>26.1±0.2†</td>
<td>25.4±0.3†</td>
</tr>
<tr>
<td>BW:BL</td>
<td>28.0±1.9</td>
<td>26.7±1.7</td>
<td>19.2±0.3†</td>
<td>18.9±0.6†</td>
</tr>
<tr>
<td>Left kidney weight, g</td>
<td>2.36±0.27</td>
<td>2.10±0.14</td>
<td>2.45±0.12</td>
<td>2.32±0.11</td>
</tr>
<tr>
<td>Right kidney weight, g</td>
<td>2.50±0.27</td>
<td>2.34±0.17</td>
<td>2.43±0.12</td>
<td>2.39±0.12</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>10.6±2.7</td>
<td>10.1±1.4</td>
<td>9.4±0.6</td>
<td>10.5±0.8</td>
</tr>
<tr>
<td>GFR, ml/min</td>
<td>1.40±0.21</td>
<td>1.53±0.13</td>
<td>1.94±0.11*</td>
<td>1.81±0.13</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>0.24±0.03</td>
<td>0.27±0.03</td>
<td>0.37±0.02*</td>
<td>0.31±0.02</td>
</tr>
<tr>
<td>Slope of gain, db/decade</td>
<td>31±5 (4)</td>
<td>47±4*</td>
<td>48±5†</td>
<td>37±3</td>
</tr>
<tr>
<td>Phase Peak, radians</td>
<td>0.99±0.13 (4)</td>
<td>1.42±0.15</td>
<td>1.60±0.15*</td>
<td>1.36±0.07</td>
</tr>
<tr>
<td>RBF, ml/min after L-NAME</td>
<td>5.2, 5.9 (2)</td>
<td>5.5±0.4 (4)</td>
<td>5.5±0.5</td>
<td>6.6±0.8</td>
</tr>
<tr>
<td>Slope after L-NAME</td>
<td>56, 46 (2)</td>
<td>47±4 (4)</td>
<td>55±7</td>
<td>48±8</td>
</tr>
<tr>
<td>Phase peak after L-NAME</td>
<td>1.94, 1.66 (2)</td>
<td>1.46±0.18 (4)</td>
<td>1.85±0.19</td>
<td>1.52±0.18</td>
</tr>
</tbody>
</table>

Data from rats after 4 wk diabetes in experiment 2 are shown. Except as noted, there were 5 intact-normal (IN-N), 5 intact-high salt (IN-H), 7 diabetic-normal (DM-N), and 7 diabetic-high salt (DM-H) rats. Body length, body weight-to-length ratio (BW:BL), left and right kidney weights, renal blood flow (RBF), glomerular filtration rate (GFR), and filtration fraction are reported in the four different treatment groups. RBF, slopes of gain, and phase peaks are reported for control and post-L-NAME periods. Least squares estimates of the slope of gain reduction by the myogenic mechanism and the amplitude of the associated phase peak are reported. *Significantly different from IN-N, P ≤ 0.02. †Significantly different from IN rats receiving the same salt regimen, P < 0.01. ‡Significantly different from IN-N, P < 0.05.

time on heart rate (both \( P < 3 \times 10^{-5} \)); the reduction due to diabetes was significant and independent of salt intake at selected time points (weeks 2, 3, 9, and 13). There was a small effect of high salt intake to reduce heart rate in intact rats at week 13 only (\( P = 0.043 \)). The dark-light difference shown in Fig. 5D displayed only a main effect of time (\( P = 10^{-5} \)), with small reductions occurring from control to week 2 in the diabetic groups (\( P = 0.007 \) in DM-N and \( P = 0.040 \) in DM-H) and between weeks 3 and 9 in IN-N (\( P = 0.027 \)).

There was no effect of increased salt intake on blood pressure in either intact or diabetic rats. Diabetic rats had higher systolic pressure at the control record (\( P = 0.013 \)), which inspection of Fig. 5E shows to arise in the DM-N group. Systolic pressure tended to rise in IN rats between the control and week 2 records (1.2 ± 0.2 and 1.2 ± 0.6 mmHg/wk in IN-N and IN-H, respectively), but not in DM rats (−0.6 ± 0.6 and 0.1 ± 0.5 mmHg in DM-N and DM-H, respectively); thus, evolution of systolic pressure differed due to diabetes (\( P = 0.012 \)). The trends established before manipulating salt intake continued during the period of high salt intake. There was a significant difference in the slope of evolution of systolic pressures between IN and DM rats (\( P = 0.00017 \)), but no effect of salt (\( P = 0.206 \)). Fig. 5F displays the dark-light difference of systolic pressure, which showed interactions between experimental condition and time (\( P = 0.045 \)) and between level of salt intake and time (\( P = 0.050 \)). These resulted largely from differences between IN-N and IN-H (\( P = 0.003 \)) and between IN-H and DM-H (\( P = 0.025 \)) at control, before any experimental manipulation. The IN-H group had lesser dark-light difference than DM-H at week 2 (\( P = 0.011 \)) and week 3 (\( P = 0.031 \)).

Renal blood flow was increased by high salt intake in diabetic rats, shown in Table 2, and numerically similar values were seen in intact rats. Steady state renal autoregulation is shown in Fig. 6. For operational reasons unrelated to experimental design or performance, data were acquired from 6 of 10 IN-N, 4 of 10 IN-H, 7 of 11 DM-N, and all 11 DM-H rats. All groups displayed canonical pressure-flow relationships with RBF being independent of perfusion pressure at pressures above 90 mmHg and dependent on perfusion pressure at lower pressures. Two way, repeated-measures ANOVA disclosed a primary effect of salt intake (\( P = 0.036 \)) on normalized RBF at renal perfusion pressures <90 mmHg, as well as the expected primary effect of perfusion pressure (\( P < 10^{-5} \)). Fig. 6 reveals that the apparent effect of salt intake was due to enhanced autoregulation at low perfusion pressure in the DM-N rats compared with all other groups.
Histological examination of the kidneys from this experiment revealed no vascular or tubular injury in any case. Glomerular injury was minor, as segmental sclerosis was observed in 2 glomeruli from an IN-N rat, in 1 glomerulus from a DM-N rat, and in 7 glomeruli from 3 DM-H rats. Focal mild mesangial expansion was seen in 5 of 11 DM-N, 5 of 11 IN-H, and in 7 glomeruli from 3 DM-H rats. Diabetic kidneys were reliably identified by the presence of PAS-positive cytoplasmic droplets in distal convoluted tubules, illustrated in Fig. 7 which shows sections from intact and diabetic rats.

**DISCUSSION**

This study addressed potential long-term consequences of diabetic hyperfiltration and its abrogation by increased salt intake. The importance of increased glomerular capillary pressure to both diabetic hyperfiltration and to nephropathy is well established (2, 6, 17, 34, 45, 46, 70). We hypothesized that diabetic hyperfiltration impairs autoregulation of RBF and thus stabilization of glomerular capillary pressure via a relative resistance shift from afferent to efferent arteriole and via net dilator influence of TGF. In the long run, this is predicted to increase renal susceptibility to diabetic and hypertensive injury. Since the putative treatment of diabetic hyperfiltration would involve increased salt intake, which may have deleterious effects of its own, two questions arise. The first, addressed here, is to determine the effects of long-term elevation of salt intake upon blood pressure and upon renal autoregulation. The second, addressed elsewhere, is to determine the effects of long-term elevation of salt intake on the incidence and rate of progression of glomerular injury.

We created a condition of poorly controlled diabetes with sustained hyperglycemia and marginal though continuous growth. The body weight-to-length ratio adequately predicts the greatly reduced lipid content that was visible in the diabetic rats (72). The rationale for insulin treatment is that untreated salt intake, elevated blood glucose.

**Fig. 4.** RBF dynamics before (A–D) and after (E–H) administration of L-NAME in experiment 2. Equivalent pressure fluctuation was achieved in all four groups both during the control period (A) and after L-NAME (E). The high coherence indicates a close linear relationship between pressure and RBF, both before (B) and after L-NAME (F). Control transfer function depicts admittance gain (C) and admittance phase (D). Local maxima in gain are seen at ~0.2 Hz and at ~0.04 Hz in all cases. The gain reduction to <0dB at frequencies below these two maxima, and the associated phase peaks, indicate effective autoregulation mediated by the myogenic mechanism (0.06 to 0.2 Hz) and by tubuloglomerular feedback (TGF) (<0.04 Hz). No diabetes-dependent differences are evident, although both slope and phase peak were less in the IN-N group than in IN-H or DM-N rats. IN-N (n = 4) is shown in light blue; IN-H (n = 5) is shown in dark blue; DM-N (n = 7) is shown in magenta; and DM-H (n = 7) is shown in red. G and H: transfer function acquired after L-NAME is shown. Robust signature of both autoregulatory mechanisms are present after L-NAME, although no modulation of the myogenic mechanism was visible in IN-H, DM-N, or DM-H. IN-N (n = 2), IN-H (n = 4), DM-N (n = 7), and DM-H (n = 7).

**Fig. 5.** Evolution of body weight (A), blood glucose (B), heart rate (C), systolic blood pressure (D), heart rate difference (E), and systolic blood pressure difference (F) from the initial control record through induction of diabetes (weeks 1 and 2), and 11 weeks of increased salt intake (denoted by the heavy black bar on the abscissa). A: diabetes, but not high salt intake, greatly slowed weight gain. B: similarly, diabetes, but not high salt intake, elevated blood glucose. C: Diabetes, but not high salt intake, caused a prompt and consistent reduction of heart rate (P < 3.10−5). D: the dark-light difference of heart rate (ΔHR, min−1) occurred between control and week 2 in DM-N (P = 0.007) and DM-H (P = 0.027). E: systolic pressure tended to rise with time in the intact rats, whereas in the diabetic rats, it was either stable (DM-H) or declined with time (DM-N). F: Dark-light difference of systolic pressure (ΔSP, mmHg) differed at control between IN-H and IN-N (P = 0.005) and between IN-H and DM-H (P = 0.025). IN-H rats also had less dark-light difference than DM-H at weeks 2 (P = 0.011) and 3 (P = 0.031). IN-N (n = 9) is denoted by the solid light blue line and solid diamonds; IN-H (n = 9) is denoted by the dashed dark blue line and open diamonds; DM-N (n = 11) is denoted by the solid magenta line and solid triangle; DM-H (n = 11) is denoted by the dashed red line and open triangles.
streptozotocin-induced diabetes in rats results not only in severe hyperglycemia but also in starvation. Data in the literature suggest that the presence (6, 69) or absence (74) of suboptimal insulin treatment does not affect the evolution of blood pressure. On the other hand, Hashimoto et al. (28) showed preservation of autoregulation in diabetic rats receiving suboptimal insulin treatment, but not in untreated diabetic rats, suggesting that starvation may compromise vascular regulation in long-term experiments. Similarly, the Long-Evans rat was used because it is the parent strain of the Otsuka Long-Evans Tokushima fatty (OLETF) rat. Male OLETF rats reliably develop type II diabetes and exhibit classic diabetic nephropathy, including nodular lesions of glomeruli (80, 81, 94). We expected that the parent strain would also show classic diabetic nephropathy. We confirmed the presence of diabetic hyperfiltration and showed that a moderate increase of salt intake reduced GFR to the same level as in intact rats, indicating that the range of salt intake was adequate to address the questions proposed.

At the end of the long protocol (experiment 3), histological examination of kidneys from intact and diabetic rats revealed a very minor renal phenotype in the diabetic rats, consistent with a previous report that glomerular injury did not differentiate control and diabetic Sprague-Dawley rats 35–40 wk into the disease (6). There was no glomerular injury (glomerulosclerosis or mesangial expansion) that identified diabetic kidneys, nor were there tubular or vascular lesions. However, diabetic kidneys were reliably identified by the presence of PAS-positive droplets in the cytoplasm of distal tubular cells, which we interpret to indicate that these kidneys were excreting protein. Because autoregulation was effective, blood pressure was unchanged, and GFR and filtration fraction were normal (at least in the high-salt rats), this observation is most easily explained by a glomerular abnormality that is unrelated to blood pressure.

**Evolution of BP in STZ diabetic rats.** The literature is ambiguous concerning blood pressure to be expected in diabetic rats. Different groups have reported no change (20, 49, 59, 82) and increased (8, 68) or decreased (37, 51) blood pressure subsequent to induction of diabetes by alloxan, streptozotocin, or pancreatectomy. Only recently have results from long-term studies tended to converge upon no change or a small reduction of blood pressure, perhaps due to acquisition of 24-h blood pressure records by telemetry. These studies have shown that blood pressure is either stable (27, 32, 74) or progressively reduced (6, 69) following induction of type 1 diabetes and that the reduction of blood pressure can be sustained over at least 35 wk (6). Consistent with these recent reports, we observed that the diabetic rats exhibited a modest and variable, although significant, reduction of systolic pressure attributable to diabetes.

Superficially, the literature appears more consistent concerning the effect of dietary salt on blood pressure in diabetes. However, there is still considerable divergence partly due to differences in the amount of salt ingested. A small change in salt load limits one’s ability to detect a salt-dependent difference, while too high a salt intake may reduce food or fluid intake and corrupt the experiment. In rats eating standard chow, salt intake is 1% of food intake by weight; in intact rats eating ~35 g/day of chow that is ~6 meq/day and ~9 meq/day in diabetic rats eating ~53 g/day of chow. Previously, modest salt sensitivity of blood pressure has been reported in intact and diabetic rats receiving >14 meq/day dietary salt (49); delayed salt sensitivity has been reported in diabetic rats receiving 8% (~72 meq/day) salt (60); salt sensitivity has been reported in diabetic rats receiving 4–5% (~40 meq/day) salt (68). On the other hand, Brands’ group found no salt sensitivity of blood pressure in diabetic rats receiving 0.07 or 12 meq/day salt, even in the presence of underlying L-NAME-induced hypertension (7).

In both intact and diabetic rats, salt was added to the drinking fluid so that the “high-salt” rats ingested salt equal to 2.5% by weight of food intake in experiment 2 (~15 and ~22 meq/day in intact and diabetic rats). Salt intake was increased to 2% in the first leg of experiment 3 and to 3.5% in the second leg (~20 and ~32 meq/day in intact and diabetic rats). In both experiments, fluid intake increased when salt was added, showing that the salt concentrations used were in the range, which rats prefer to water (58, 66) and providing assurance that the experiments were not confounded by aversive responses. Similar assurance is provided by the chronic renal function curve, which documents the independence of blood pressure from salt intake within the range of intake used here.

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**Table 2. Results from increased dietary salt in rats after 13 wk of diabetes**

<table>
<thead>
<tr>
<th></th>
<th>IN-N</th>
<th>IN-H</th>
<th>DM-N</th>
<th>DM-H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length, cm</td>
<td>27.5±0.5</td>
<td>27.0±0.4</td>
<td>26.2±0.2†</td>
<td>25.8±0.2†</td>
</tr>
<tr>
<td>Left kidney weight, g</td>
<td>2.14±0.14</td>
<td>1.98±0.17</td>
<td>2.29±0.10</td>
<td>2.43±0.04*</td>
</tr>
<tr>
<td>Right kidney weight, g</td>
<td>2.02±0.20</td>
<td>2.02±0.18</td>
<td>2.58±0.12*</td>
<td>2.47±0.07*</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>9.3±2.0</td>
<td>11.9±1.4</td>
<td>8.4±1.1</td>
<td>13.2±1.6</td>
</tr>
</tbody>
</table>

Data from rats after 13 wk of diabetes in experiment 3 are shown. Body length, BW:BL, left and right kidney weights, and renal blood flow in the four different treatment groups (intact rats receiving normal and high salt diet, diabetic rats receiving normal and high salt diet) are reported. *Significantly different from IN, P < 0.05. †Significantly different from IN rats receiving the same salt regimen, P < 0.01. ‡Significantly different from DM-N, P < 0.05.

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**Fig. 6. RBF normalized to the flow at 90 mmHg as a function of perfusion pressure.** In all four groups, RBF at pressures ≥90 mmHg was independent of perfusion pressure. As shown in the inset, autoregulatory indices (90 to 100 mmHg) were not different from zero in any group, indicating highly efficient autoregulation. When renal perfusion pressure was reduced below 90 mmHg, RBF declined progressively. However, in the DM-N rats, the decline was significantly less than in the other three groups, which were indistinguishable. †Significantly different between DM-N and DM-H. ‡Significantly difference between DM-N and IN-N. n = 6, 4, 7, 11 for IN-N, IN-H, DM-N, DM-H respectively.
Thus, among the four groups, salt intake was varied in total over a 4- to 5-fold range for an extended period and under conditions that would be expected to expose salt sensitivity of blood pressure (49). To our knowledge, this is the first study to examine the effect of salt intake on blood pressure in long-term studies of diabetic rats, while using telemetric data acquisition. The study was performed in three experiments with the long protocol performed in two cohorts. They were controlled internally with an initial control record acquired prior to any experimental manipulation and externally with contemporaneous intact rats subject to the same dietary manipulation. In none of them were we able to identify any effect of added salt on 24-h average systolic pressure in the intact or diabetic rats.

Although 24-h systolic pressure is clearly independent of salt intake, others have reported instances in which salt sensitivity could be detected in the dark phase, but not in 24-h records (9). Such a finding would be consistent with preferential intake during the dark phase when rats are more active. Although such differences were seen in the long experiment, all arose from the control record and were therefore irrelevant to the experiment. Thus, we were unable to detect any salt sensitivity of blood pressure and must conclude that in rats with poorly controlled diabetes, increasing salt intake to a level sufficient to abrogate diabetic hyperfiltration is a null event as downstream reabsorption, this would tend to correct any propensity to retain salt. In fact, recent work suggests that distal sodium reabsorption may be blunted in diabetic rats (57). Thus, both morphological and physiological data are consistent with retained ability to excrete a salt load rapidly and quantitatively.

This study and many others show reduced heart rate in diabetic rats, mice, and rabbits e.g., (8, 11, 19, 32, 37, 47, 49, 52, 59, 61). The reduction occurs at the onset of diabetes and results from increased parasympathetic traffic to the heart (27, 51a). It is rapid, substantial, and consistent. In contrast, the reduction of systolic pressure tends to occur slower and is more variable in magnitude, shown by differing trajectories of systolic pressure in the two groups within each of two experiments. Our data do not provide an explanation for this variability, but do highlight its existence and caution against overinterpretation of the hypotensive effect.

**RBF autoregulation in diabetic rats.** In a series of studies, Vallon and colleagues (77, 83) developed and tested a “tubulocentric” view of diabetic hyperfiltration. They showed that fractional proximal reabsorption is inversely related to salt intake in diabetic rats, so that raising salt intake reduces proximal reabsorption and increases solute concentrations in fluid reaching the macula densa, with resulting preglomerular vasoconstriction. This view has been challenged in two studies employing adenosine type 1 receptor knockout (or “TGFless”) mice. Although these groups reported diabetic hyperfiltration in the absence of TGF (18, 67), a recent report indicates that TGFless mice display neither diabetic hyperfiltration nor the salt paradox (87). The design of the current study was based upon the tubulocentric construction. However, even if diabetic hyperfiltration were to have some other etiology, it does result in glomerular hypertension, and this is corrected by a modest increase of salt intake. Thus, the importance of autoregulation to understanding consequences of changing salt intake in diabetes would be unchanged.

It is commonly considered that autoregulation is impaired in diabetes mellitus (10, 30, 36). However, the finding of impaired autoregulation is by no means uniform (13, 24, 28, 29, 51), and the literature has been described as “contradictory”(6, 13). Similarly, RBF dynamics have been evaluated only three times, but even so with conflicting results (4, 5, 24). Development of STZ-induced diabetes results in increased macula densa nNOS (76, 93), which has particular relevance to autoregulation (35, 71, 91) and might be expected to limit the effectiveness of autoregulation and RBF dynamics. Yet our results strongly support effective autoregulation in diabetes mellitus.
We examined renal autoregulation using both steady-state pressure ramps and assessment of RBF dynamics, with forcing of blood pressure, and with nonselective inhibition of NOSs. In two experiments, the steady-state pressure-flow relationship showed equally effective autoregulation in intact and diabetic rats when systemic pressure was above ~90 mmHg. Furthermore, autoregulation was extended to lower perfusion pressure only in diabetic rats receiving normal salt intake, indicating that autoregulation is actually improved in the diabetic rats. Assessment of RBF dynamics often picks up subtle changes that may be missed in steady-state autoregulation experiments (15, 89). RBF dynamics in the IN-N rats are typical of those seen historically in normotensive strains with mixed first- and second-order dynamics (90). In contrast, the IN-H rats and both diabetic groups show fully second-order dynamics indicating highly efficient autoregulation. The current results are consistent with data acquired in conscious ZSF1 rats, which showed unchanged RBF dynamics compared with lean controls (24). Thus, the results of steady-state and dynamic experiments consistently showed strong autoregulation in the diabetic rats.

The classic modulation of the myogenic mechanism by inhibition of NOSs involves a switch from first- or mixed-first- and second-order dynamics to fully second-order (41, 71, 90). The response to increased availability of nitric oxide is impaired pressure-induced constriction (38, 39), while increased cyclic GMP reduces slope of gain reduction and amplitude of the phase peak (92). Because our diabetic rats and IN-H rats displayed high slopes of gain reduction and high-phase peaks, it is not surprising that there was little or no response of RBF dynamics to inhibition of NOSs. The interpretation is clearly that the kidneys of all groups except perhaps the intact rats on normal salt intake were in a low nitric oxide state.

The steady state experiment detected a salt-sensitive left shift in diabetic rats, while the dynamics results are most consistent with a low nitric oxide condition. Both the left shift and the hyperfiltration are abrogated by elevated salt intake. The left shift of the lower limit of autoregulation has been repeatedly observed in diabetic rats (16, 50, 51) and has been attributed to an action of nitric oxide (78). This conclusion, although reasonable at the time it was drawn, is difficult to reconcile with current understanding of the role of nitric oxide in renal autoregulation. First, nonselective inhibition of NOSs causes a pronounced left shift of the lower limit of autoregulation (31, 43, 44, 64, 79). Second, inhibition of NOSs enhances autoregulation within the normal autoregulatory range of renal perfusion pressure (44, 64, 79). Third, both the left shift and hyperfiltration were seen (DM-N) and abrogated by high salt intake (DM-H) in rats in which we were unable to detect modulation of RBF dynamics by nitric oxide. The most parsimonious explanation would be that changing salt intake affects both and by a common mechanism. Thus increased salt intake results in normalized salt concentration at the macula densa, which will reduce renin secretion. On balance, we suggest that ANG II may be important in generating the left shift. ANG II modulates both autoregulatory mechanisms (42, 63), and a functional renin-angiotensin system is required for hypotension to induce left shifts of autoregulation (14, 33, 55, 73). Furthermore, renal interstitial nitric oxide concentration is reduced in diabetes and the reduction is ANG II dependent (3).

The data concerning autoregulation in the current study are internally consistent between methods and among experiments. They show that autoregulation is well preserved in early and chronic diabetes mellitus and that increased dietary salt has only a minor effect on autoregulation. The results suggest that nitric oxide plays at most a minor role in modulating autoregulation in early diabetes. In particular, they are not consistent with a role for nitric oxide in mediating the resetting of autoregulation to operate at lower renal perfusion pressure. That said, we recognize a recent study, also in STZ diabetic rats, having diametrically opposed results (4) and do not have a good explanation for the difference.

In summary, diabetic changes were reliably detected by telemetry (reduced heart rate and systolic pressure), by renal functional studies (increased GFR and left-shifted autoregulation), and by renal histology (PAS-positive droplets in distal tubule cells). However, neither telemetry nor histology detected any pathological effect of increased dietary salt intake either alone or in combination with diabetes. Effects of high salt were seen only in renal functional studies as restoration of GFR and of renal autoregulation toward the control state. In conclusion, under the conditions of the current study, we were not able to identify any salt sensitivity of blood pressure induced by diabetes. Nor was it possible to identify any independent or synergistic effects of increased dietary salt on renal function or structure in diabetes.

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GRANTS

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