Chronic Sodium-Retaining Action of Insulin in Diabetic Dogs

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ABSTRACT

Insulin-mediated sodium retention is implicated as a mechanism for hypertension in metabolic syndrome and Type II diabetes. However, there is no direct experimental evidence for a sustained antinatriuretic effect of insulin outside of rodents, and all previous studies in dogs have been negative. This study used a novel approach to test for a chronic sodium retaining action of insulin in dogs, by testing the hypothesis that natriuresis in Type I diabetes is dependent on the decrease in insulin, rather than being due solely to osmotic actions of hyperglycemia. Dogs were chronically instrumented and housed in metabolic cages. Fasting blood glucose in alloxan-treated dogs was maintained at ~65 mg/dl by continuous iv. insulin infusion. Then a 6-day diabetic period was induced by either: 1) decreasing the insulin infusion to induce Type I diabetes (D; blood glucose = 449±40 mg/dl), or 2) clamping the insulin infusion and infusing glucose continuously (DG; blood glucose = 470±56 mg/dl). Control urinary sodium excretion (UnaV) averaged 70±5 (D) and 69±5 (DG) mEq/day, and increased on day 1 in both groups. UnaV remained elevated in the D group (115±15 mEq/day days 2-6), but returned to control in the DG group (69±11 mEq/day days 2-6) and was accompanied by decreased lithium clearance. Thus, insulin had a sustained antinatriuretic action that was triggered by increased glucose, and it was powerful enough to completely block the natriuresis caused by hyperglycemia. These data may reveal an unrecognized physiologic function of insulin as a protector against hyperglycemia-induced salt wasting in diabetes.
INTRODUCTION

Early observations in diabetic human subjects showed acute natriuresis following abrupt withdrawal of insulin therapy and antinatriuresis upon resumption of insulin therapy, suggesting that insulin could affect renal sodium excretion (2). Subsequently, DeFronzo et al (17) showed that acute insulin infusion in euglycemic human subjects significantly decreased urinary sodium excretion due most likely to stimulation of tubular reabsorption. Numerous studies have supported that finding by showing significant decreases in urinary sodium excretion during acute insulin infusion in animals (18, 22, 30, 36) and humans (20, 46, 48), and insulin-mediated sodium retention has been hypothesized to cause hypertension, particularly in obese subjects (16, 19, 31, 39-41, 44, 50).

However, the only chronic data that support a sodium-retaining or hypertensive action come from rodent studies (10, 11, 35, 47, 51). In fact, chronic insulin infusion studies in dogs have not found any evidence for a direct sodium retaining effect (12, 24-26). Therefore, it has been exceedingly difficult to translate the results from acute insulin infusion studies and chronic rat studies that support a sodium-retaining hypothesis to any chronic sodium-retaining action in humans.

To address this problem and test whether insulin has a direct renal sodium retaining action in dogs, we developed a unique experimental approach. Typical insulin infusion studies achieve hyperinsulinemia while simultaneously maintaining normal plasma glucose levels, i.e. a euglycemic clamp. Although that is a sound experimental design to isolate insulin as an independent variable, it creates a condition that does not mimic the insulin-glucose relationship found either in Type I diabetes (which is low insulin and very high glucose) or in metabolic
syndrome and Type II diabetes (which is a continuum of elevated insulin and glucose together, varying with disease progression and severity). Our approach, on the other hand, allowed us to determine the impact of the change in insulin that actually accompanies the pathologic state. We hypothesized that if insulin has a chronic sodium-retaining action, then the sustained natriuresis caused by induction of Type I diabetes could be mediated, at least in part, by the decrease in baseline insulin levels rather than being due solely to an osmotic diuretic effect of glucose. This was tested by comparing the renal sodium excretory responses in two groups of diabetic dogs with no difference in their level of hyperglycemia: one with low plasma insulin typical of Type I diabetes, and one in which plasma insulin did not change from baseline levels.

MATERIALS AND METHODS

Studies were conducted in conditioned male mongrel dogs weighing approximately 25 kg and all experimental protocols were approved by the Institutional Animal Care and Use Committee of the Medical College of Georgia. A flow probe (3PSB; Transonic, Ithaca, NY) was placed on the left renal artery and the right kidney was removed. A Data Sciences (DSI St. Paul, MN) TA11PA-D70 blood pressure unit was implanted in the right femoral artery, and standard fluid-filled Tygon catheters were implanted in the right femoral vein in the left femoral artery and vein. The catheters and probe cable were tunneled subcutaneously to the scapular region and exteriorized, and the dogs were fitted with a polypropylene jacket equipped with a pocket to hold the catheters and flow probe cable.
Following recovery, dogs were placed in individual metabolic cages and connected to a customized electrical/hydraulic swivel unit that enabled continuous intravenous infusion and electrical connectivity while the dogs had completely unrestricted, 360-degree freedom of movement in the cage 24 h/day. Sodium intake was maintained constant (i.e. clamped) at an average of 81±3 mEq/day for the D group and 83 ± 2 mEq/day for the DG group by feeding a low-sodium diet (Hills H/D; 3, 13 oz cans per dog per day) coupled with a continuous intravenous infusion of approximately 475 ml of 0.9% saline per day, every day throughout the study. In addition, all dogs received approximately 975 ml of sterile water vehicle per day, and drinking water was available ad libitum.

Every day at 0800 hours fasting blood glucose was measured in all dogs. At 1100 hours every day throughout the study the "daily routine" began, which consisted of the following activities always in the same order: dogs were fed, infusion solutions were measured and replaced, 24-hour water intake was measured, and 24-hour urine volume was measured and sampled. On GFR measurement days, serial blood samples were drawn between 0800 and 1100 hours, thus not affecting the timing of the regular daily routine. Veterinary staff evaluated the dogs every day at variable times between 0800 and 1400 hours, and cage cleaning was completed before 1400 hours. Blood pressure and renal blood flow (RBF) measurement began at 1400 hours and continued through to 0800 hours the next day. (It was limited to this 18-hour window because of the variable activity (blood samples, vet checks, room mechanical maintenance) between 0800 and 1400 hours. The blood pressure and flow signals were sampled for 10 seconds each minute at 100 Hz using the A.R.T. software from DSI. Approximately 2
weeks were allowed for the dogs to acclimate to the metabolic cages and be trained to lie quietly for blood sampling.

Experimental Procedure

To prevent insulin from decreasing during diabetes, we first used alloxan (50 mg/kg) to decrease endogenous insulin in all dogs so that we could control plasma insulin chronically throughout the experiment. To avoid potential renal complications from concentrating alloxan, the iv. saline infusion was increased to 1000 ml/day for 2 days preceding alloxan administration, and each dog was given mannitol (50 ml of a 250 mg/ml solution, iv.) 30 minutes immediately preceding alloxan administration. Blood glucose reached diabetic levels 1-3 days after alloxan, at which point dogs were placed on insulin replacement therapy. Regular insulin was infused iv. 24 hr/day, and the dose was adjusted daily in each dog based on daily fasting blood glucose measurement (21 hrs postprandial). After stable blood glucose had been achieved (~1 week), control period measurements were begun. This was followed by a 6-day diabetic period in which similar levels of hyperglycemia were induced in 2 groups of dogs by one of two methods: 1) standard Type I diabetes was induced by decreasing the insulin replacement dose (D; Diabetes - low insulin, n=11), or 2) an insulin-clamp model in which the control period insulin replacement dose for a given dog was continued unchanged, and glucose was infused iv. to create hyperglycemia (DG; Diabetes – iv glucose, n=9). The DG dog glucose infusion was accomplished by replacing the sterile water vehicle with a 50% dextrose solution, and the rate of glucose infusion was adjusted based on daily blood glucose measurement. Dogs were assigned randomly to their groups. After 6 days of diabetes, control conditions were resumed in both groups and normal glucose levels were restored.
Analytical Procedures

Fasting blood samples (21 hrs postprandial) were drawn during the control period, on diabetes days 2 and 5, and during the recovery period in all dogs in both groups. On the same days, glomerular filtration rate (GFR) was determined from the total plasma clearance of $^{125}$-labeled iothalamate (Glofil; QOL Medical, Kirkland, WA) over a 3-hour period from 0800-1100 while the dogs rested quietly in their cages (9, 27). Urine sodium, potassium, and lithium concentrations were determined by atomic absorption, plasma electrolytes were measured by ion-sensitive electrodes (MEDICA Easy Electrolytes, Bedford, MA), plasma protein concentration was measured by refractometry, blood glucose was measured with an Accu-Check meter (Roche, Indianapolis, IN), urine glucose was measured using a glucose assay kit (Sigma-Aldrich, St. Louis, MO), osmolality was measured by freezing point depression (Advanced Instruments, Norwood, MA), plasma insulin was measured using an EIA kit from ALPCO Diagnostics (Salem, NH), and plasma renin activity (PRA) was measured by radioimmunoassay (Diasorin, Stillwater, MN). Daily electrolyte and water balances were calculated as: intake - output - insensitive loss, where insensitive loss equaled average intake during the control period - average output during the control period. Therefore, cumulative balance equals zero on the last day of the control period.

Data from D and DG dogs were analyzed with 2-factor repeated measures analysis of variance (ANOVA) using GraphPad Prism software. At $p < 0.05$ for the within-subjects F-test in the ANOVA, Dunnett’s test was used to determine which experimental-period day differed from control. The control for time in this experimental design is the recovery period, and for variables with multiple recovery period days, an average recovery period value was determined and used
in the ANOVA. At \( p < 0.05 \) for the between-subjects F-test in the ANOVA, bonferroni test was used to determine on which days the two groups differed. Statistical significance versus control was \(^* p<0.05\), and for the DG versus the D group \(^# p<0.05\). All data are expressed as mean ± SEM.

RESULTS

Glucose and Insulin

All dogs in this study were treated with alloxan, and all dogs were placed on daily insulin replacement (Insulin Rx) to maintain normal blood glucose. Glucose was measured once per day under fasting conditions in all dogs at 0800 hours to minimize variability. These dogs were assigned randomly to either the D or DG groups, and Figure 1 shows that the 24 hr/day Insulin Rx dose was stable and not different between groups during the baseline period. Figure 2 shows that fasting blood glucose also was not different between groups at baseline. Thus, there was no difference in the, surgery, housing, preparation, daily care, or treatment of dogs in the D vs. DG group prior to the 6-day diabetic period, and this is supported by the baseline insulin and glucose data.

Diabetes was induced in the D group by decreasing the insulin replacement dose (Figure 1, gray bars). The decrease in plasma insulin is shown in Table 1, and the increase in blood glucose that resulted is shown in Figure 2 (gray bars). Therefore, the D group had typical Type I diabetes, with low insulin and high blood glucose. In the DG group, the control-period insulin replacement dose was clamped at that rate and maintained (Figure 1, black bars) while hyperglycemia was induced by infusing glucose iv. 24 hr/day. The glucose infusion was
adjusted as needed in each dog to raise blood glucose to the same level as in the D group (Figure 2, black bars).

Fasting blood glucose averaged 64 ± 4 and 68 ± 8 mg/dl in the D and DG groups, respectively, during the last 3 days of control (Figure 2), and increased significantly in both groups and not differently between groups over the 6-day diabetic period, averaging 449 ± 40 and 470 ± 56 mg/dl in the D and DG groups, respectively. It was important that the glucose infusion rate in the DG dogs did not have to be increased during the diabetic period to maintain the elevated blood glucose levels, because that would have indicated there was glucose-induced insulin release occurring in the background; therefore, this was additional verification that endogenous insulin secretion did not increase in response to the glucose infusion. Table 1 shows that plasma osmolarity increased to similar levels in both groups during the diabetic period, which reinforces the similar levels of hyperglycemia measured.

Sodium Excretion and Fluid Balance

Urinary sodium excretion was not different between groups during the control period (Figure 3), and total water intake averaged 2194 ± 163 and 2170 ± 77 ml/day in the D and DG groups, respectively. On the first day of diabetes urinary sodium excretion increased significantly in both groups (Figure 3), and that is consistent with the early measurements of increased urinary osmolar clearance (Table 2) and decreased extracellular fluid volume (ECFV, Table 1) in both groups. However, there was a dramatic divergence in the sodium and volume excretory responses from the second diabetic day onward, with urinary sodium excretion increasing further and being sustained in the D dogs, but with the natriuresis being virtually abolished in the DG dogs despite no differences in blood glucose concentration and sodium
intake being clamped at the same level in both groups. This is consistent with the D group having significantly greater urine osmole excretion (Table 2) and significantly lower plasma sodium concentration (Table 1) than the DG group, and also having a significant increase in renal sodium clearance (Table 2). The differences in cumulative sodium balance (Figure 4) show the integrated consequences of these effects over the 6-day period.

The sodium excretion data are consistent with the continued decrease in ECFV in the D group but not the DG group (Table 1). Water intake increased approximately 200 ml/day more in the D group than in the DG group by day 6 of diabetes, but cumulative water balance was more negative in that group (Table 1), and the differences in cumulative water balance approximated the differences in ECFV. Those changes in volume status are consistent with a tendency for hematocrit and plasma protein concentration (Table 1) to increase in the D group but not the DG group. Thus, renal sodium clearance and indexes of ECFV and plasma volume all are consistent with the urinary sodium excretion data showing that the natriuresis during diabetes was not sustained beyond one day if insulin was not allowed to decrease.

Tubular Reabsorption

Table 2 shows that lithium clearance in the DG group decreased significantly during diabetes and returned to normal during the recovery period. Figure 5 shows that fractional lithium reabsorption increased significantly during diabetes in the DG group, but decreased transiently in the D group. These data are consistent with increased proximal tubular sodium reabsorption in the DG dogs during the diabetic period. Our initial measurements of urinary glucose excretion showed that it was significantly lower in the DG versus D dogs during diabetes, averaging 335 ± 114 and 251 ± 118 mg/day on days 2 and 5 of diabetes, respectively,
for DG, and 551 ± 161 and 604 ± 166 mg/day on days 2 and 5, respectively, for D. However, that range from approximately 250 to 600 mg/day is considerably lower than the approximately 65 to 260 grams/day previously reported using acute urine collection in diabetic dogs (14, 45). We measured urine glucose concentration at the conclusion of the study from samples stored under refrigeration, and after obtaining these low values we analyzed small frozen aliquots of urine we had saved from 4 dogs in each group. That analysis showed 24-hour urinary glucose excretion to be in the 20 gram/day range. The finding of lower excretion in DG vs. D dogs was preserved, and those are the data shown in Table 2. Plasma renin activity (PRA) increased on diabetes day 2 in the DG dogs (Table 1), suggesting a potential role for the renin angiotensin system in the early-stage sodium retention. The renal blood flow (RBF, Figure 6) and GFR (Table 2) responses showed no evidence of vasoconstriction, which also supports a tubular mechanism for the sodium retention. Mean arterial pressure averaged 104 ± 4 and 105 ± 2 mmHg in the D and DG dogs, respectively, and did not change during diabetes in either group, and because there also were no differences in GFR (Table 2) or RBF between groups, those hemodynamic variables cannot explain the different sodium excretion responses between groups.

DISCUSSION

Increased urinary sodium excretion is a hallmark of Type I diabetes, and we hypothesized that preventing the decrease in insulin would attenuate the increase in urinary sodium excretion, thereby providing the first direct experimental evidence for a chronic sodium retaining action of insulin in a non-rodent model. Surprisingly, however, we found that maintaining plasma insulin at baseline levels completely abolished the natriuresis after one day and returned sodium
excretion to normal despite persistent hyperglycemia. This was shocking in light of the classic textbook dogma that an osmotic diuretic effect of glucose in diabetes is responsible for the tremendous urinary salt and volume losses (5, 23, 52), so much so that persistent hyperglycemia is believed to cause "massive salt and water depletion, and cardiovascular collapse may ensue." (52) Yet we did not measure any progressive salt depletion, decreased extracellular fluid volume, or decrease in blood pressure in the DG group. Therefore, not only do these data provide direct evidence for a sustained sodium retaining effect of insulin that is linked to hyperglycemia, but they also challenge a long-held concept regarding the mechanism for renal salt and water loss in diabetes.

The marked natriuresis on day 1 of diabetes of course is consistent with that concept, and strongly suggests that hyperglycemia was the mediator of the initial, transient, rise in sodium excretion. Because RBF was not increased on day 1 of diabetes, the natriuresis that day likely was not a consequence of renal vasodilation. This is consistent with two recent findings from our laboratory: First is that chronic blockade of nitric oxide synthesis prevented the increases in GFR and RBF caused by onset of Type I diabetes in rats (consistent with other reports (32, 49)), but did not attenuate the natriuretic response (6). Second is that induction of Type I diabetes in rats with reduced kidney mass, in which GFR was decreased 50% at baseline and did not increase during diabetes, caused significant natriuresis that was not different from rats with normal kidney mass and increased GFR (42). These data support the natriuretic effect of hyperglycemia in diabetes.

However, the sodium excretory responses measured on diabetes days 2-6 show that the sustained natriuresis during Type I diabetes was dependent entirely on the decrease in insulin, because maintenance of baseline insulin levels was able to counteract the natriuretic effect of
sustained hyperglycemia. Numerous studies have shown that insulin infusion or administration can decrease sodium excretion or increase tubular sodium transport (10, 11, 18, 20, 22, 30, 35, 46-48, 51). However, all studies that have shown direct antinatriuretic actions of insulin have been acute studies in humans or other species or chronic studies in rodents, and the sodium retention reported previously during chronic iv. insulin infusion dogs was indirect, due to the drop in blood pressure and withdrawal of pressure natriuresis (12, 25, 26). That was reinforced by the lack of sodium retention during chronic intra-renal insulin infusion in dogs (24). A critical difference in our approach in this study is that we did not infuse insulin to induce hyperinsulinemia, but instead tested the role of the insulin depletion that occurs Type I diabetes. As presented in the Methods, our only blood samples for measuring insulin, as well as glucose, were 21 hours postprandial, and whether those variables changed differently between groups in response to the daily meal is not known. Therefore, our strongest evidence that our experiment tested the role of insulin depletion is the significant difference in 24-hour insulin replacement doses shown in Figure 1. Maintenance of the control-period insulin replacement dose during hyperglycemia in the DG group showed that the loss of insulin is what enables persistent hyperglycemia to cause sustained natriuresis, thereby revealing a sustained sodium retaining action of insulin.

Most evidence for insulin-induced antinatriuresis implicates action in the distal nephron (17, 18, 20, 47, 48), possibly through modulation of ENaC or sodium-chloride cotransport (47). There also is evidence that insulin can stimulate chloride transport in the loop of Henle (30) and increase sodium-potassium ATPase (18) and sodium-hydrogen exchanger activity (21, 22) in the proximal tubule. The decrease in lithium clearance and increase in fractional lithium reabsorption (Figure 5) in the DG group are consistent with a proximal tubular site of action.
However, the measurement of decreased fractional lithium reabsorption in the D group was surprising in light of evidence that proximal fractional sodium reabsorption is increased in diabetes (34, 54-56). In fact, we previously have invoked this as a mechanism that contributes significantly to renal vasodilation in diabetes, by causing the macula densa to sense a decrease in sodium chloride delivery (6, 7). This mechanism for withdrawal of tubulogomerular feedback (TGF) has been proposed by other laboratories as well to explain renal vasodilation (54-56), and we used transfer function analysis to show blunting of TGF feedback gain in conscious diabetic rats (6, 7). Because the current results were at odds with these other findings, we also estimated proximal fractional sodium reabsorption in both groups from our electrolyte and osmolar excretion data according to the following formula: 

\[
(100 - \frac{[\text{U}_{\text{Na+K}} \times V]}{\text{GFR} \times P_{\text{Na}}} \times 100\% - \frac{[\text{CH}_2\text{O} \times \text{GFR}]}{100\%})
\]

(55), and we obtained the same changes as shown by lithium clearance. We cannot explain why we saw no evidence for increased fractional proximal sodium reabsorption in the D group. These measurements during the first 5 days of diabetes are not possible in humans and to our knowledge have not been reported in chronically instrumented dogs, so whether different results would have been measured in longstanding diabetes or at a different level of hyperglycemia is not known. Likewise, we cannot rule out a role for more distal nephron sites in mediating the sodium reabsorption. In addition, a limitation inherent in chronic animal studies is that 24 hour electrolyte excretion must be coupled with single-point GFR values to calculate electrolyte clearance and fractional excretion. Nevertheless, these data support a role for proximal tubular reabsorption contributing to sodium reabsorption during diabetes in the DG group.

It is interesting, to consider a potential effect in the proximal tubule in this experimental model, because insulin was studied in the context of hyperglycemia rather than under euglycemic
clamp. Glucose already is known to increase absolute proximal tubular sodium reabsorption in diabetes through sodium-glucose cotransport (SGLT) (3, 37, 54, 57), and our data showing decreased urinary glucose excretion in the DG versus the D dogs, together with increased lithium reabsorption in the DG dogs, is consistent with increased SGLT action. In addition, serum- and glucocorticoid-inducible kinase (SGK1), typically linked to the distal nephron (8), recently has been shown to regulate proximal tubular glucose transport (1), be stimulated by glucose (1, 43) and insulin (28, 33), and to regulate NHE3 activity in the proximal tubule (21). Therefore, an intriguing question is whether an effect of glucose to stimulate proximal tubular sodium reabsorption was potentiated by maintenance of baseline insulin, possibly via SGK1-driven SGLT and/or NHE3 activity. Although that mechanism is speculative at this point, our data nonetheless suggest that insulin and glucose may have acted together to stimulate proximal sodium reabsorption.

Evidence supporting a cooperative effect of insulin and glucose to stimulate sodium reabsorption is that fractional lithium reabsorption increased significantly in the DG group. If the maintenance of normal insulin in the DG group simply had "blocked" the natriuretic effect of hyperglycemia, then fractional lithium reabsorption should have remained flat at baseline levels and tracked parallel to total urinary sodium excretion. In other words, urinary sodium excretion in the DG group returned to control levels, but it was accompanied by increased fractional lithium reabsorption. This not only is indicative of increased proximal tubular sodium reabsorption, but it also means that hyperglycemia continued to exert a natriuretic effect during the diabetic period, or else the increased sodium reabsorption caused by insulin would have increased cumulative sodium balance, rather than simply preventing the decrease. In addition, the stimulation of proximal reabsorption that occurred when the kidneys of the DG dogs were
presented with increased glucose suggests that hyperglycemia may have acted in concert with
insulin, or "triggered", an effect of insulin to stimulate sodium reabsorption. Thus, the chronic
sodium-retaining action of insulin was powerful enough to counteract the glucose-induced
natriuresis, but it appears actually to require diabetic levels of hyperglycemia to become active.

The sodium retaining action did not increase blood pressure, and that may be because the
net effect of that action was to prevent a hyperglycemia-induced decrease in cumulative sodium
balance. GFR also did not change significantly in either group, which is peculiar given the
general association of increased GFR with Type I diabetes. A potential explanation is that GFR
was measured over a 3-hour period that started 21 hrs from the last meal, and thus does not
incorporate postprandial changes that may have occurred. Blood samples from all dogs are taken
always while they are resting quietly in the post-absorptive or fasting state to minimize
variability, so this is an inherent limitation that results in a short window for GFR measurement
and only on selected days during the study. Therefore, we developed the ability to measure RBF
continuously throughout the day as one approach to measure renal hemodynamics over a longer
period, not only throughout each day, but every day during the study (9). In fact we used a
similar RBF method in rats to show that GFR and RBF increase in parallel in Type I diabetes (7).
Renal blood flow in these dogs did increase modestly; however, the change was transient. This
suggests that our GFR results may not have been due solely to the conditions under which it was
measured. GFR and RBF have been reported to be elevated in alloxan-induced diabetes in dogs
1 or more years after induction (13, 29), but we are not aware of any previous studies reporting
GFR and RBF in Type I diabetic dogs during the first week of induction, and there are very little
renal function data in conscious subjects during the first week of diabetes in any species (7, 53).
Although an explanation for the renal hemodynamic response is not apparent, hemodynamics are important to consider because changes in sodium excretion or clearance can reflect differences in filtered sodium due to renal vasoconstriction and/or sodium reabsorption, which also can have a vasoconstrictor component. Our continuous RBF measurements were critical, therefore, in providing evidence that renal vasoconstriction did not cause the sustained return of sodium excretion to normal in the DG group. For example, because GFR was measured only over a 3-hr period twice during the diabetic period, we cannot rule out that the decrease in urinary sodium excretion on diabetes D2 in the DG group was not due in part to a combination of afferent arteriolar dilation and efferent arteriolar constriction (PRA was elevated on D2), that increased GFR, maintained RBF, and therefore increased filtration fraction. However, the 18-hour daily RBF data, that show sustained normal sodium excretion with an increase in RBF on subsequent days, argue strongly against renal vasoconstriction mediating that sodium retaining action. Therefore, although not ruling out any role for vasoconstriction, the fact that there were no differences in MAP, GFR, or RBF between the two groups suggests it is unlikely that hemodynamic mechanisms explain the markedly different sodium excretory responses between the two groups over the 6-day diabetic period.

Thus, the fundamental observation in this study is that sustained hyperglycemia, over 400 mg/dl for 6 days in dogs, did not cause sustained natriuresis when circulating insulin was maintained at baseline levels. Our results do not exclude potential systemic actions of insulin, because insulin is known to stimulate the sympathetic nervous system (4, 15, 38), and there likely were differences in insulin-dependent glucose uptake in insulin-sensitive tissues between the D and DG groups as well. Nonetheless, our data are consistent with sustained stimulation of renal tubular sodium reabsorption by insulin, possibly in the proximal tubule. In addition, that
effect appears to be due to interaction between insulin and tubular glucose and to be powerful enough to oppose a sustained natriuretic influence of hyperglycemia. The renin angiotensin system may have been involved during the first few days, and the possibility that insulin and glucose affected sodium transport by SGLT, perhaps via SGK1, is interesting to consider. It also is intriguing to extrapolate from these findings and consider whether protecting against glucose-induced renal sodium loss is a normal, physiologic function of insulin that simply has not been recognized previously. Such an action might be operative postprandially, for example, but it also may mean that maintenance of sodium balance during the progression of metabolic syndrome and uncontrolled Type II diabetes, in which plasma insulin and glucose both are elevated chronically, is due to a cooperative effect of insulin and glucose in the renal tubule to counteract and prevent progressive sodium loss from the sustained hyperglycemia. That hypothesis, however, will require further study.
ACKNOWLEDGEMENTS

The authors acknowledge the technical assistance of Tuere Sheppard and Ashlyn Allen. This work was supported by the National Heart Lung and Blood Institute, HL56259.


FIGURE LEGENDS

**Figure 1.** Insulin infusion dose in the D group (n=11; shaded bars) and DG group (n=9; black bars) during insulin replacement treatment (Insulin Rx) in the control (C) and recovery (R) periods, and during the diabetic period (D or DG). Alloxan (allox) was administered 1-2 weeks before control period data were collected. Data shown as mean ± SEM. * p<0.05 vs. control; # p<0.05 vs. D.

**Figure 2.** Blood glucose in the D group (n=11; shaded bars) and DG group (n=9; black bars) during insulin replacement treatment (Insulin Rx) in the control (C) and recovery (R) periods, and during the diabetic period (D or DG). Alloxan (allox) was administered 1-2 weeks before control period data were collected. Data shown as mean ± SEM. * p<0.01 vs. control.

**Figure 3.** Urinary sodium excretion in the D group (n=11; shaded bars) and DG group (n=9; black bars) during insulin replacement treatment (Insulin Rx) in the control (C) and recovery (R) periods, and during the diabetic period (D or DG). Alloxan (allox) was administered 1-2 weeks before control period data were collected. Data shown as mean ± SEM. * p<0.05 vs. control, # p<0.05 vs. the D group.

**Figure 4.** Cumulative sodium balance in the D group (n=11; shaded bars) and DG group (n=9; black bars) during insulin replacement treatment (Insulin Rx) in the control (C) and recovery (R)
periods, and during the diabetic period (D or DG). Alloxan (allox) was administered 1-2 weeks before control period data were collected. Data shown as mean ± SEM. * p<0.05 vs. control, and # p<0.05 vs. the D group.

Figure 5. Fractional lithium reabsorption in the D group (n=11; shaded bars) and DG group (n=9; black bars) during insulin replacement treatment (Insulin Rx) in the control (C) and recovery (R) periods, and during days 2 and 5 of the diabetic period (D or DG). Alloxan (allox) was administered 1-2 weeks before control period data were collected. Data shown as mean ± SEM. * p<0.05 vs. control, and # p<0.05 vs. the D group.

Figure 6. Renal blood flow in the D group (n=11; shaded bars) and DG group (n=9; black bars) during insulin replacement treatment (Insulin Rx) in the control (C) and recovery (R) periods, and during the diabetic period (D or DG). Alloxan (allox) was administered 1-2 weeks before control period data were collected. Data shown as mean ± SEM. * p<0.05 vs. control.
Table 1. Plasma composition and extracellular fluid volume.

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<th>Day 5</th>
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<td>309 ± 2</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>310 ± 2</td>
<td>321 ± 3 *</td>
<td>321 ± 3 *</td>
<td>313 ± 5</td>
</tr>
<tr>
<td>Hct</td>
<td>D</td>
<td>36 ± 2</td>
<td>40 ± 1 *</td>
<td>39 ± 2</td>
<td>38 ± 2</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>40 ± 2</td>
<td>35 ± 1</td>
<td>37 ± 3</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>P Protein</td>
<td>D</td>
<td>6.7 ± 0.2</td>
<td>7.6 ± 0.1</td>
<td>7.6 ± 0.1</td>
<td>7.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>6.9 ± 0.2</td>
<td>7.0 ± 0.2</td>
<td>7.2 ± 0.1</td>
<td>6.5 ± 0.2</td>
</tr>
<tr>
<td>ECFV</td>
<td>D</td>
<td>7754 ± 669</td>
<td>6785 ± 803</td>
<td>6428 ± 456 *</td>
<td>6859 ± 340 *</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>7380 ± 373</td>
<td>6924 ± 360</td>
<td>6970 ± 254</td>
<td>7346 ± 462</td>
</tr>
</tbody>
</table>

P Insulin = plasma insulin (uU/ml), PRA = plasma renin activity (ng AngI/ml/hr), P Na+ = plasma sodium (mEq/L), P K+ = plasma potassium (mEq/L), P Osm = plasma osmolality (mOsm/kg), Hct = hematocrit (%), P Protein = plasma protein (g/dl), ECFV = extracellular fluid volume (ml). Data are mean ± sem, * p<0.05 vs. control, # p<0.05 vs. D group. D group n = 11 and DG group n = 9.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Control</th>
<th>Day 2</th>
<th>Day 5</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR</td>
<td>D</td>
<td>50 ± 5</td>
<td>48 ± 6</td>
<td>47 ± 4</td>
<td>51 ± 3</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>48 ± 3</td>
<td>52 ± 3</td>
<td>53 ± 4</td>
<td>49 ± 5</td>
</tr>
<tr>
<td>( U_{\text{osm}} )</td>
<td>D</td>
<td>610 ± 59</td>
<td>1274 ± 111(^*)</td>
<td>1319 ± 96(^*)</td>
<td>618 ± 22</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>647 ± 55</td>
<td>811 ± 122(^*) #</td>
<td>946 ± 192(^*) #</td>
<td>720 ± 69</td>
</tr>
<tr>
<td>( U_{K^+} )</td>
<td>D</td>
<td>52 ± 3</td>
<td>75 ± 3 (^*)</td>
<td>59 ± 5</td>
<td>52 ± 2</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>54 ± 3</td>
<td>53 ± 13</td>
<td>55 ± 7</td>
<td>57 ± 8</td>
</tr>
<tr>
<td>( U_{\text{Glucose}} )</td>
<td>D</td>
<td>ND</td>
<td>19 ± 6 (^*)</td>
<td>49 ± 9 (^*)</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>ND</td>
<td>12 ± 4 (^*) #</td>
<td>10 ± 3 (^*) #</td>
<td>ND</td>
</tr>
<tr>
<td>( C_{\text{Osm}} )</td>
<td>D</td>
<td>1.17 ± 0.13</td>
<td>2.76 ± 0.24 (^*)</td>
<td>2.88 ± 0.22 (^*)</td>
<td>1.92 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>1.28 ± 0.06</td>
<td>1.74 ± 0.26</td>
<td>2.04 ± 0.41</td>
<td>1.55 ± 0.19</td>
</tr>
<tr>
<td>( C_{\text{H}_2\text{O}} )</td>
<td>D</td>
<td>-0.38 ± 0.08</td>
<td>-1.48 ± 0.17 (^*)</td>
<td>-1.74 ± 0.17 (^*)</td>
<td>-0.88 ± 0.14 (^*)</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>-0.27 ± 0.09</td>
<td>-0.92 ± 0.18 (^*) #</td>
<td>-1.16 ± 0.29 (^*) #</td>
<td>-0.58 ± 0.18</td>
</tr>
<tr>
<td>( C_{\text{Li}} )</td>
<td>D</td>
<td>11.0 ± 2.8</td>
<td>30.6 ± 7.9 (^*)</td>
<td>19.1 ± 2.8 (^*)</td>
<td>24.4 ± 4.2 (^*)</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>17.4 ± 2.3 (^*) #</td>
<td>12.0 ± 1.8 (^*) #</td>
<td>10.4 ± 2.1 (^*) #</td>
<td>19.6 ± 3.1 (^*) #</td>
</tr>
<tr>
<td>( C_{\text{Na}} )</td>
<td>D</td>
<td>0.33 ± 0.02</td>
<td>0.55 ± 0.05 (^*)</td>
<td>0.63 ± 0.10 (^*)</td>
<td>0.31 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>0.32 ± 0.03</td>
<td>0.35 ± 0.07 (^*) #</td>
<td>0.27 ± 0.03 (^*) #</td>
<td>0.35 ± 0.04</td>
</tr>
<tr>
<td>( \text{Bal}_{\text{H}_2\text{O}} )</td>
<td>D</td>
<td>0</td>
<td>-830 ± 84 (^*)</td>
<td>-1155 ± 241 (^*)</td>
<td>-1272 ± 496 (^*) #</td>
</tr>
<tr>
<td></td>
<td>DG</td>
<td>0</td>
<td>-136 ± 92 (^*) #</td>
<td>-482 ± 218 (^*) #</td>
<td>-557 ± 425 (^*) #</td>
</tr>
</tbody>
</table>

GFR = glomerular filtration rate (ml/min), \( U_{\text{osm}} \) = osmolar excretion (mOsm/day), \( U_{\text{Glucose}} \) = potassium excretion (mEq/day), \( C_{\text{osm}} \) = osmolar clearance (ml/min), \( C_{\text{H}_2\text{O}} \) = free water clearance (ml/min), \( C_{\text{Li}} \) = lithium clearance (ml/min), \( C_{\text{Na}} \) = sodium clearance (ml/min), \( \text{Bal}_{\text{H}_2\text{O}} \) = cumulative water balance. Data are mean ± sem, \(^*\) p<0.05 vs. control; \(^\#\) p<0.05 vs. D group. D group n = 11 and DG group n = 9. n = 4 per group for \( U_{\text{Glucose}} \).
Figure 1

Insulin Infusion Dose (U/day) vs TIME (days) for different conditions:
1. Diabetes – low insulin
2. Diabetes – iv glucose
Figure 2

**Diabetes – low insulin**

**Diabetes – iv glucose**

**Insulin Rx**

**Blood Glucose (mg/dl)**

**TIME (days)**

- **Allox, C1, C2, C3**: Low blood glucose levels.
- **D1, D2, D3, D4, D5, D6**: Significant increase in blood glucose levels, marked with asterisks (*) indicating statistical significance.
- **R1, R2, R3**: Blood glucose levels revert to baseline.

**Insulin Rx**

- **Blood Glucose (mg/dl)**
- **TIME (days)**
- **Allox, C1, C2, C3**: Low blood glucose levels.
- **DG1, DG2, DG3, DG4, DG5, DG6**: Increase in blood glucose levels, marked with asterisks (*) indicating statistical significance.
- **R1, R2, R3**: Blood glucose levels revert to baseline.
Figure 3

- Insulin Rx
  - Diabetes – low insulin

- Insulin Rx
  - Diabetes – iv glucose

Bar charts showing urinary sodium excretion (mEq/day) over time (days) for different conditions and treatments.
Figure 4

[Graph showing cumulative sodium balance over time for different insulin regimens, with annotations for significant differences indicated by stars and hash marks.]
Figure 5

Fractional Lithium Reabsorption (%)

TIME (days)

Insulin Rx | Diabetes low insulin | Insulin Rx

![Graph showing fractional lithium reabsorption over time for different conditions.]
Figure 6

**Diabetes – low insulin**

**Diabetes – iv glucose**