Blunted Diuretic and Natriuretic Responses to Central Administration of Clonidine in Streptozocin-Induced Diabetic Rats

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The purpose of this study was to determine whether diuretic and natriuretic effects are altered in response to intracerebroventricular (ICV) infusion of clonidine in diabetic rats. Diabetes was induced in male Sprague-Dawley rats by 65 mg/kg i.p. injection of streptozocin, and control rats were injected with vehicle 2 wk before the experiment. Blood glucose levels were significantly elevated in the diabetic group (26.3 ± 1.3 mM) compared with the control group (8.4 ± 1.6 mM). Before and during ICV infusion of clonidine (2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 45 min), urine flow and sodium excretion were measured from intact and denervated kidneys in anesthetized diabetic and control rats. The ICV infusion of clonidine significantly increased urine flow in both innervated and denervated kidneys from control rats but not from diabetic rats. There was a significant increase in sodium excretion during ICV infusion of clonidine from innervated kidneys of control rats, and denervation abolished this effect. In diabetic rats, clonidine failed to promote natriuresis from intact kidneys, and similar to control rats, did not promote natriuresis in denervated kidneys. This study demonstrates that *1*) the diuretic response to the ICV infusion of clonidine is blunted in diabetic rats, and *2*) a natriuretic response to the ICV infusion of clonidine is blunted in innervated kidneys of diabetic rats.
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Diabetes mellitus is characterized by polyuria, polydipsia, extracellular fluid hyperosmolality, and a reported increase in intravascular volume (1–3). We have demonstrated that an acute volume expansion produced a blunted diuresis and natriuresis in streptozocin (STZ)-induced diabetic rats (4). The blunted natriuresis was attributed to a decreased renal sympathoinhibition in diabetic rats (4). Renal sympathoinhibition and natriuresis are regulated, in part, by central α_2-receptors (5,6). Combining this with the fact that diabetic rats were reported to have altered noradrenergic activity in central sites (7–9) involved in the volume reflex (6,10,11), we reasoned that the central α_2-mechanism may be altered in diabetic rats to produce the blunted volume reflex. The α_2-agonist clonidine causes diuresis and natriuresis (12–14). Diuresis in response to intracerebroventricular (ICV) administration of clonidine is attributed to inhibition of release and/or the peripheral action of antidiuretic hormone (13,15). We have observed that natriuresis in response to ICV administration of clonidine is dependent on the activity of renal nerves (14).

The purpose of this study was to determine *1*) whether diuresis in response to ICV infusion of clonidine was altered in diabetic rats and *2*) whether natriuresis regulated by renal nerves was blunted in response to ICV infusion of clonidine in diabetic rats. If these responses are blunted, then it would be apparent that normal noradrenergic α_2-mechanisms may be impaired in the diabetic state.

RESEARCH DESIGN AND METHODS

Experiment 1. Male Sprague-Dawley rats (200–250 g, Sasco-King, Omaha, NE) were assigned randomly to either a control or diabetic group. They were placed in individual cages in a room with a controlled 12-h light-dark cycle and maintained at 20–22°C. Food and water were available ad libitum. Diabetes was induced by a single injection of STZ (65 mg/kg i.p., Sigma, St. Louis, MO) in a 2% solution of cold 0.1 M citrate buffer (pH 4.5). Onset of diabetes occurred rapidly and was identified by polydipsia, polyuria, and blood glucose concentrations >13.9 mM 2 days after injection of STZ. Blood glucose was measured by a blood glucose monitor (Accu-Chek II, Boehringer Mannheim, Indianapolis, IN). Control rats received a similar volume of vehicle (citrate buffer) alone. Two weeks after the injection of STZ or vehicle,

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TABLE 1
Mean arterial blood pressure, heart rate, and blood glucose in control and diabetic rats

	Arterial pressure (mmHg)		Heart rate (beats/min)		Blood glucose (mM)
	Con	Clo	Con	Clo	
Control	107 ± 5	94 ± 4*	375 ± 15	295 ± 15*	8.4 ± 1.6
Diabetic	104 ± 4	86 ± 4*	323 ± 15†	265 ± 17	26.3 ± 1.3†

Values are means ± SE for 7 rats in each group. Con, basal period immediately before intracerebroventricular (ICV) infusion of clonidine. Clo, period during ICV infusion of clonidine.
*$P < 0.05$ vs. Con.
†$P < 0.05$ vs. control group.

the kidney function experiments were performed on each of the rats.

On the day of the experiment, rats were anesthetized with Inactin (0.1 g/kg i.p.). Body temperature was maintained between 36 and 38°C via external warming by a heated stage. After tracheal intubation, the animals were allowed to breathe independently. The left femoral artery was cannulated with PE-50 tubing and connected to a pressure transducer (Gould P23 ID, Oxnard, CA) for the continuous recording of arterial pressure (Grass polygraph, model 7D, Quincy, MA). Heart rate was measured with a tachygraph that was triggered by the arterial pulse. Both blood pressure and heart rate averaged over 15-min periods are reported. After the left femoral vein was cannulated with PE-50 tubing, a constant isotonic saline infusion (20 μl/min) was started.

For kidney denervation and ureteral cannulation, the kidneys were exposed through an abdominal incision. Denervation of the left kidney was performed by stripping the sheath and adventitia from the exposed left renal artery and vein. To destroy any remaining nerve fibers, the kidney vessels were painted with 95% ethanol. Then, both ureters were cannulated with PE-10 tubing. Kidney denervation has been shown to decrease the kidney norepinephrine concentration to <5% of control (16). In addition, urine output from the denervated kidney was consistently greater than from the contralateral innervated kidney. Such a preparation will have increased renal nerve activity to the intact kidney because of both Inactin anesthesia (17) and the renorenal reflex (18). However, this effect is not expected to alter the renal sympathoinhibition that should occur in response to ICV administration of clonidine.

On the day of the experiment, to measure the renal response to central administration of clonidine, the rats were placed in a stereotaxic apparatus. An injection cannula was lowered through a small trephined hole into the lateral cerebral ventricle. The injection cannula was constructed of a 30-gauge stainless steel hypodermic needle. Clonidine infusion (2 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in 2 μl/min of vehicle [isotonic saline]; Sigma) was administered through the injection cannula connected by PE-10 tubing to a 5-ml syringe driven by an infusion pump (model 355, Orion, Boston, MA). All surgery was completed within 75 min, and an additional 30-min stabilization period was allowed before the start of the first urine collection.

After two 15-min basal urine collections, urine was collected every 15 min during the 45-min ICV infusion of clonidine (2 $\mu g \cdot kg^{-1} \cdot min^{-1}$). This dose of clonidine has been demonstrated to produce a robust diuresis and natriuresis (12,14). Although this dose may be slightly high, it was chosen to ensure that reliable and large diuretic and natriuretic responses occurred in the control rats. After measuring the urine volume gravimetrically, the sodium content (ion-selective electrode, Beckman ion analyzer, Irvine, CA) and osmolality (5100 vapor pressure osmometer, Wescor, Logan, UT) of the urine samples were analyzed.

Experiment 2. The purpose of this study was to determine whether the introduction of a mere saline vehicle into the ICV space in diabetic rats over the time frame of experiment 1 was responsible for the attenuated kidney responses to ICV

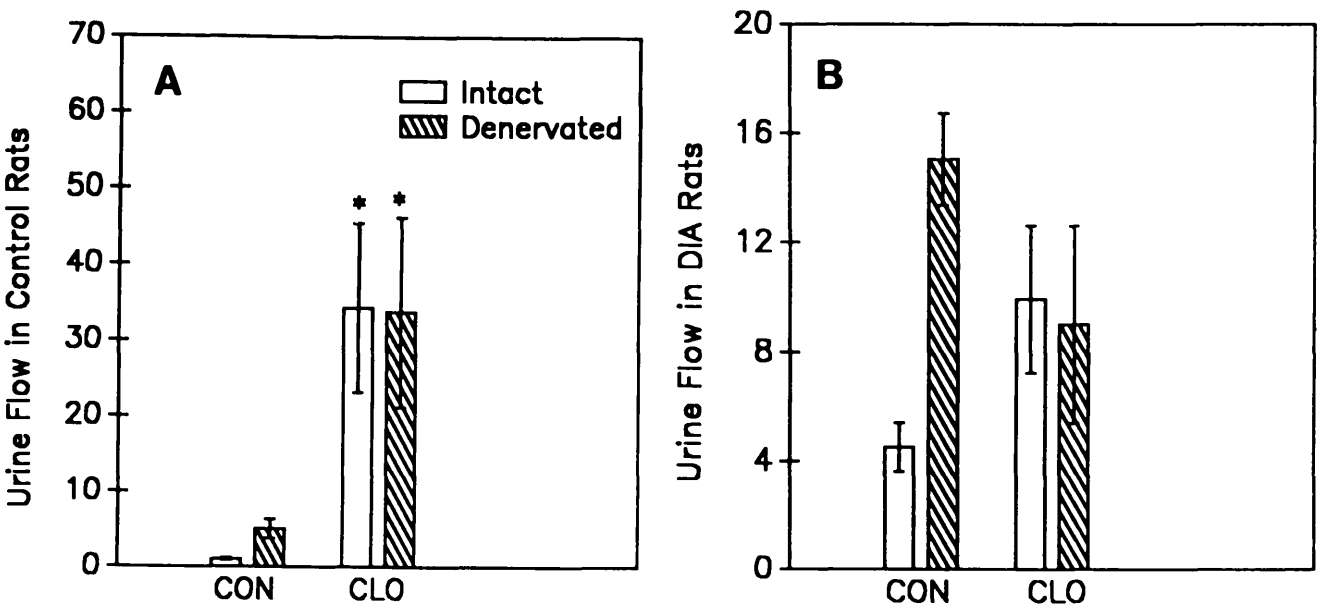

FIG. 1. Urine flow ($\mu l \cdot min^{-1} \cdot g^{-1}$ kidney wt) during 15-min period just before clonidine infusion (CON) and during last 15-min infusion of clonidine (CLO) from intact and denervated kidneys in control (*A*; $n = 7$) and diabetic (DIA; *B*; $n = 7$) groups. Values are means ± SE. *$P < 0.05$ vs. CON.

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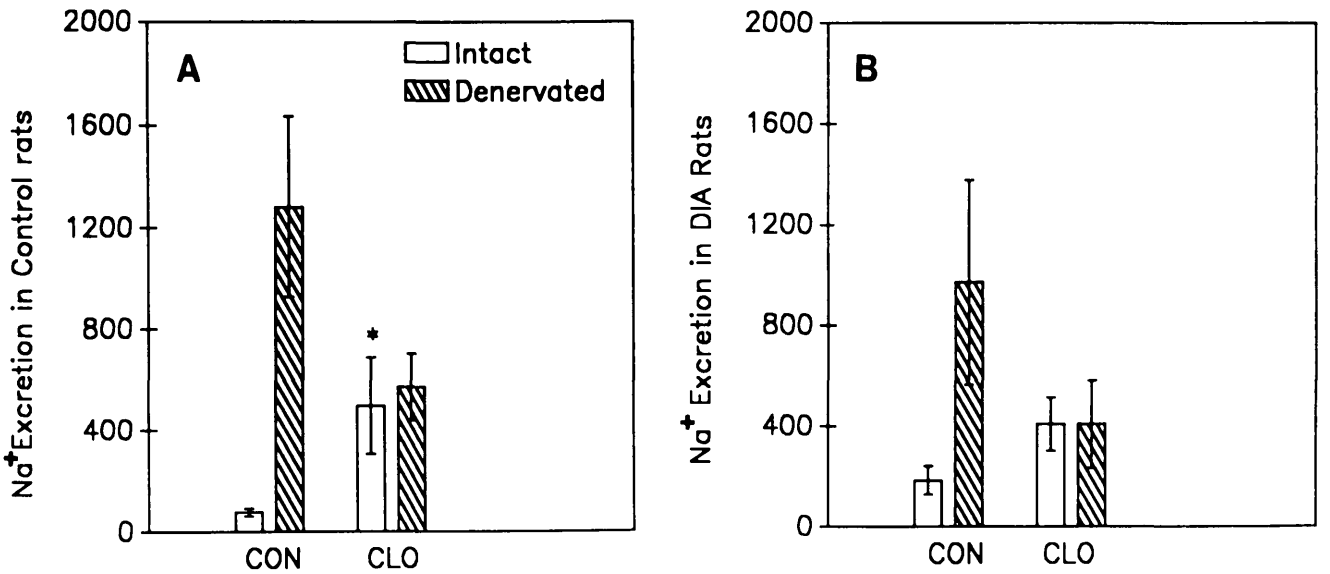

FIG. 2. Sodium excretion (neq · min^{-1} · g^{-1} kidney wt) during 15-min period just before clonidine infusion (CON) and during last 15-min administration of clonidine (CLO) from intact and denervated kidneys in control (*A*; *n* = 7) and diabetic (DIA; *B*; *n* = 7) groups. Values are means ± SE. *$P < 0.05$ vs. CON.

introduction of clonidine in diabetic rats. Diabetic rats were assigned randomly to either a vehicle group (group 1) or a clonidine group (group 2). The rats were treated as in experiment 1, except that, in experiment 2, rats from group 1 were infused with the vehicle (isotonic saline) into the ICV space at the same flow rate (2 μl/min for 45 min).

Data analysis. The results of the basal period (15 min) immediately before and during the ICV infusion of clonidine (3rd 15-min collection) in the diabetic or the control groups were compared. Significant differences in blood pressure, heart rate, urine flow, sodium excretion, and urine osmolality before and during the ICV infusion of clonidine were evaluated with Student's *t* test for dependent means (19). Blood pressure, heart rate, and blood glucose data were subjected to Student's *t* test for independent means to assess the differences between the control and diabetic groups in the experiments (19). All data are expressed as means ± SE. $P < 0.05$ was statistically significant.

RESULTS

Experiment 1. Mean arterial blood pressure in the diabetic group was not significantly different from the control group before or during the ICV infusion of clonidine (Table 1). Clonidine significantly lowered the mean blood pressure in both diabetic and control groups compared with their respective basal mean blood pressures (i.e., before clonidine infusion). Heart rate was significantly slower in the diabetic group compared with the control group before the ICV infusion of clonidine. However, there was no difference in heart rate between the control and diabetic groups during the ICV infusion of clonidine (Table 1). Clonidine significantly reduced heart rate in the control rats but not in the diabetic rats. Blood

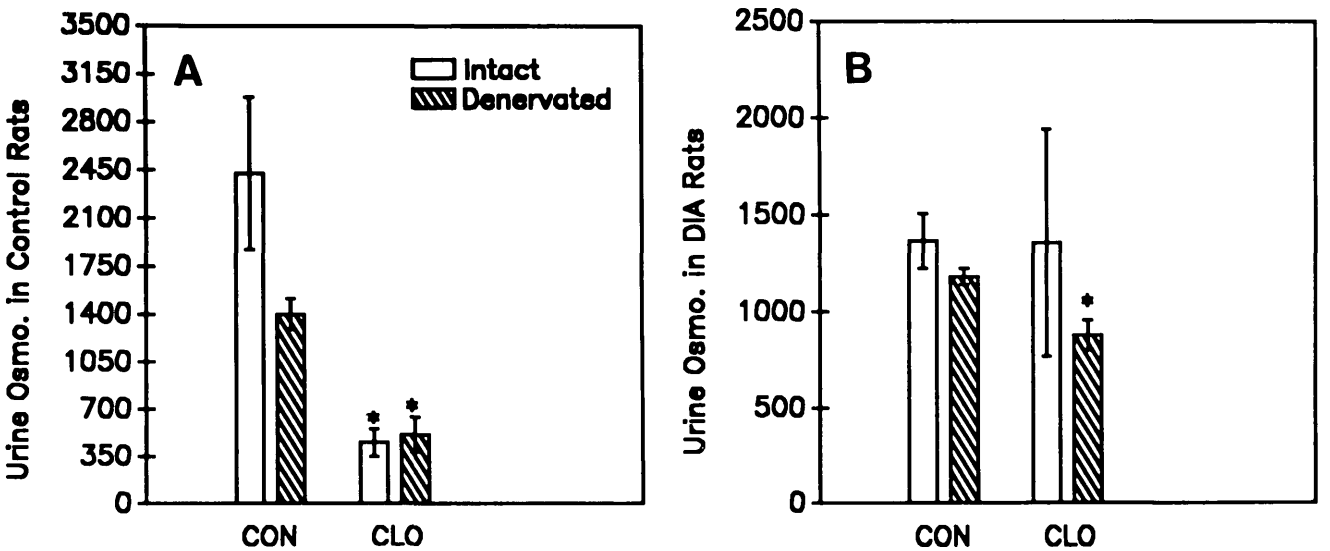

FIG. 3. Urine osmolality (mmol/kg) during 15-min period just before clonidine infusion (CON) and during last 15-min administration of clonidine (CLO) from intact and denervated kidneys in control (*A*; *n* = 7) and diabetic (DIA) (*B*; *n* = 7) groups. Values are means ± SE. *$P < 0.05$ vs. CON.

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TABLE 2
Mean blood pressure and blood glucose in diabetic time-control and diabetic groups

	n	Arterial pressure (mmHg)		Blood glucose (mM)
		Pretreatment	Treatment	
Diabetic time control	8	93 ± 5	93 ± 7	23.4 ± 1.2
Diabetic	6	89 ± 6	80 ± 7	23.6 ± 1.5

Values are means ± SE. There were no significant differences.

glucose levels were significantly higher in diabetic rats compared with control rats as expected (Table 1). All diabetic rats had a blood glucose level ≥19.4 mM at the time of the experiment.

Data on kidney responses to ICV infusion of clonidine are presented for the basal period (15 min) immediately before the ICV infusion of clonidine and then the period during the ICV infusion of clonidine (3rd 15-min collection). In previous studies, these periods have been used reliably to determine the changes in kidney responses to various perturbations (e.g., ICV administrations of clonidine) (4,6,14). We have also observed no significant changes in urine flow or sodium excretion over the time frame of this experiment (time control) in previous studies on nondiabetic control rats (unpublished observations).

In control rats, there was a significant increase in urine flow from both intact and denervated kidneys in response to the ICV infusion of clonidine (Fig. 1*A*). In regard to natriuresis, a significant increase was seen only from the kidneys of control rats with the renal nerves intact, and denervation abolished this effect (Fig. 2*A*). However, clonidine caused a significant decrease in osmolality of urine from both intact and denervated kidneys (Fig. 3*A*).

In diabetic rats, no significant diuresis was observed from innervated or denervated kidneys (Fig. 1*B*). Clonidine failed to promote natriuresis from innervated kidneys and, similar to control rats, did not promote natriuresis in denervated kidneys either (Fig. 2*B*). There were no significant differences of urine osmolality from intact kidneys, but there was a statistical decrease in osmolality of urine from the denervated kidney in response to the ICV infusion of clonidine (Fig. 3*B*).

Experiment 2. Mean blood pressure was not significantly different between the two groups of rats before or during treatment (Table 2). In addition, blood pressure in group 1 (diabetic time-control rats) remained constant before and during ICV infusion of vehicle, whereas clonidine reduced blood pressure by 9 mmHg in group 2 (Table 2). The two groups had similar high levels of blood glucose (Table 2).

Urine flow and sodium excretion from innervated and denervated kidneys in group 1 (diabetic time-control rats) were not changed by the ICV infusion of vehicle alone (Figs. 4*A* and 5*A*). There were also no significant differences in osmolality of urine from kidneys in group 1 either before or during the administration of vehicle into the ICV space (Fig. 6*A*). Results from experiment 2 with diabetic rats indicate that ICV infusion of vehicle over 45 min is not a critical factor per se in altering blood pressure, urine flow, or sodium excretion. Similarly, we have also observed no significant changes in urine flow or sodium excretion over the time frame of the experiment (time control) in previous studies on nondiabetic control rats (unpublished observations).

Although clonidine increased urine flow and sodium excretion from innervated kidneys of diabetic rats, these changes were not statistically significant (Figs. 4*B* and 5*B*). There were no statistically significant differences in diuresis and natriuresis from denervated kidneys in response to ICV infusion of clonidine (Figs. 4*B* and 5*B*). There were also no differences in the osmolality of urine from both intact and denervated kidneys of group 2 in response to clonidine (Fig.

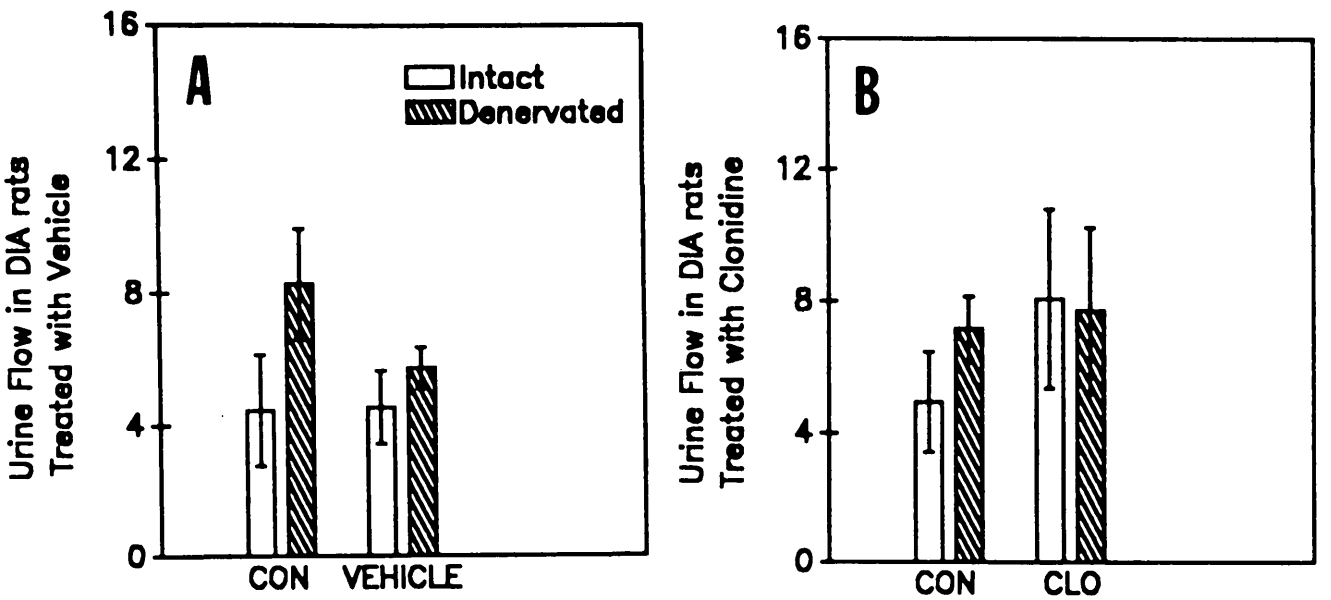

FIG. 4. Urine flow (μl · min^{-1} · g^{-1} kidney wt) during 15-min period just before treatment (CON) and during last 15-min infusion of vehicle or clonidine (CLO) from intact and denervated kidneys in diabetic (DIA) time-control (*A*; *n* = 8) and diabetic (*B*; *n* = 6) groups. Values are means ± SE. *$P < 0.05$ vs. CON.

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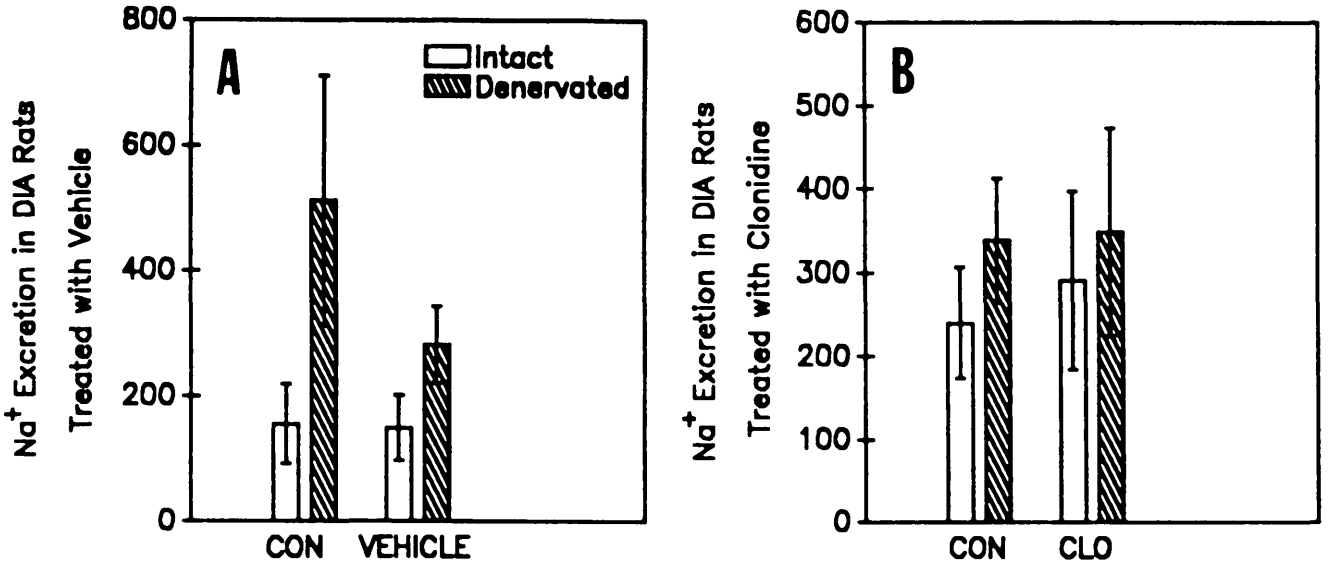

FIG. 5. Sodium excretion (neq · min^{-1} · g^{-1} kidney wt) during 15-min period just before treatment (CON) and during last 15-min infusion of vehicle or clonidine (CLO) from intact and denervated kidneys in diabetic (DIA) time-control (*A*; *n* = 8) and diabetic (*B*; *n* = 6) groups. Values are means ± SE. **P* < 0.05 vs. CON.

6*B*). Basically, these results are similar to those observed in diabetic rats administered clonidine in experiment 1.

DISCUSSION

Increased levels of circulating antidiuretic hormone have been reported to occur in diabetes in both humans and rats (20,21). This may be due to a hyperosmolality (mainly hyperglycemia) of extracellular fluid. Although there is an increased intravascular volume in diabetic rats (1–3), a change in osmolality of ≥2% has a greater capacity to stimulate the release of antidiuretic hormone than a 5% change of blood volume (22). Thus, the increased antidiuretic hormone in diabetic rats is understandable. Inhibition of antidiuretic hormone release is suggested to be a major factor by which clonidine produces diuresis (13,15). In this study, a reduced inhibition of the release of antidiuretic hormone in response to the central effect of clonidine may be responsible for the observed decreased diuresis in diabetic rats compared with control rats. The chronically high levels of antidiuretic hormone in the plasma of diabetic rats (e.g., 9.8 ± 1.7 pg/ml) might not be reduced to as low a level as in the control rats (e.g., 2.3 ± 0.5 pg/ml) by the inhibitory action of clonidine (20). One possible explanation for the reduced inhibition of the release of antidiuretic hormone may be downregulation of α_2-receptors or decreased α_2-receptor–effector coupling. Another possibility may be related to altered noradrenergic mechanisms in the forebrain, specifically the hypothalamus (supraoptic and paraventricular nucleus) of diabetic rats (7–9). These areas in the CNS are involved in the regulation of fluid balance (10). In this study, clonidine might not have been effective in inhibiting the release of antidiuretic hormone in diabetic rats and, thus, produced a reduced diabetic response in diabetic rats.

We have reported that natriuresis produced by clonidine

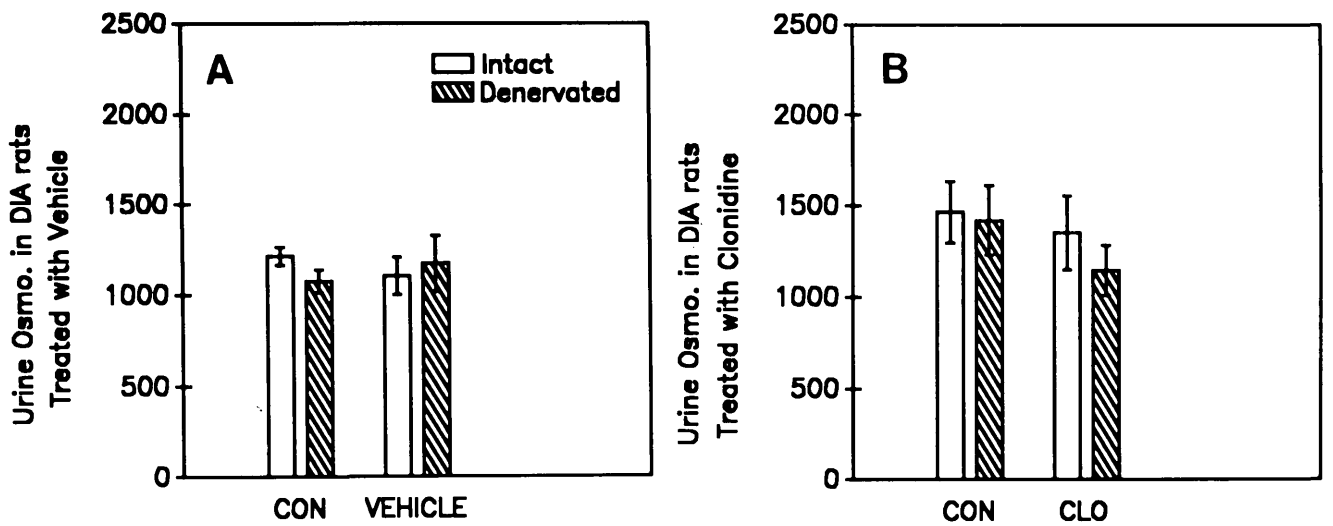

FIG. 6. Urine osmolality (mmol/kg) during 15-min period just before treatment (CON) and during last 15-min infusion of vehicle or clonidine (CLO) from intact and denervated kidneys in diabetic (DIA) time-control (*A*; *n* = 8) and diabetic (*B*; *n* = 6) groups. Values are means ± SE. **P* < 0.05 vs. CON.

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was dependent on the acitivity of renal nerves (14). This is supported by studies showing that renal nerve activity was decreased when clonidine was administered centrally (5). The lack of a natriuresis from the innervated kidneys of diabetic rats may be due to a blunted renal sympathoinhibition in response to clonidine. Note that the decrease in heart rate during the ICV infusion of clonidine was smaller in diabetic than control rats, further supporting a blunted sympathoinhibition in diabetic rats. Because central α_2-adrenergic mechanisms contribute to the CNS processing of the volume reflex (6), the results of this study may provide an explanation for the blunted renal sympathoinhibition in response to volume expansion in diabetic rats reported previously (4). Although speculative, it would be of interest to examine whether centrally acting antihypertensive agents (α_2-agonists) are less effective in diabetic patients.

It is possible that centrally administered clonidine may leak out of the ICV space (via the blood-brain barrier; 23) and have a peripheral action directly on the kidneys (13). Therefore, in this study, the blunted diuresis in response to ICV infusion of clonidine in the diabetic rats might be attributed, in part, to the blunted diuretic action of clonidine directly on the kidney. Further investigation is needed to determine the importance of the possibility that a peripheral action of clonidine is responsible for the reduced diuresis observed in diabetic rats.

Clonidine is used clinically as an antihypertensive drug (24). In experiment 1, blood pressure was reduced in both groups during ICV infusion of clonidine. Lower blood pressure in diabetic rats (86 vs. 94 mmHg in control group), although not statistically different, may functionally influence the urine flow and sodium excretion. However, urine flow and sodium excretion from intact kidneys of control rats increased five- to sixfold in response to the ICV infusion of clonidine, whereas mean values for these parameters were increased only twofold in diabetic rats (experiment 1). It is not likely that such differences can be attributed totally to a statistically insignificant difference in blood pressure. Therefore, blood pressure per se may not be a major factor in the reduced diuresis and natriuresis in response to the ICV infusion of clonidine in the diabetic rats. In addition, neither blood pressure nor renal excretory parameters were changed in the time-control study with diabetic rats (experiment 2). Therefore, the reduced diuresis and natriuresis in diabetic rats may be mainly due to their blunted response to clonidine.

In summary, this study demonstrates that the ICV administration of clonidine produces *1*) a blunted diuresis in the diabetic rats possibly due to a reduced inhibition of antidiuretic hormone release and/or action and *2*) a blunted natriuresis in the diabetic rats that may be related to decreased renal sympathoinhibition. These results suggest that there may be a defect in the central neural processing leading to diuresis and natriuresis in diabetic rats. These data could be interpreted to suggest that this potential mechanism may be involved in the blunted volume reflex previously observed in diabetic rats (4). Consequently, this central defect may be part of the reason for the altered fluid balance observed in the diabetic state.

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