Renal Nerves Are Involved in the Natriuresis and Diuresis Produced by Central Administration of Clonidine in the Rat (43515)

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Abstract. **To determine whether renal nerves are involved in natriuresis or diuresis produced by the intracerebroventricular administration of clonidine (0.2, 2.0, and 8 μg/kg/min, and 2.0 μl/min), urine flow, and sodium excretion were measured before and during clonidine administration from innervated and contralateral denervated kidneys in anesthetized (Inactin, 0.1 g/kg, ip) Sprague-Dawley rats. Baseline urine flow and sodium excretion were elevated after renal denervation prior to infusion of clonidine. Examining urine flow and sodium excretion before and during clonidine infusion indicated significant increases in urine flow and sodium excretion from the innervated kidneys but not from the denervated kidneys, possibly due to the renal sympatho-inhibition in the innervated kidney. However, the higher doses of clonidine (2 and 8 μg/kg/min) may have diffused out of the intracerebroventricular space into the peripheral circulation and produced their effect by a direct action on the kidney. Subsequently, two experiments were performed to distinguish between a central action and peripheral action. First, clonidine was administered centrally with concurrent administration of an α2-blocker, yohimbine (8 μg/kg/min, iv), peripherally. In a second experiment the dose of clonidine was reduced 10-fold such that this reduced dose did not produce a peripheral action but still produced the renal responses to central administration. The results of the latter two studies further confirmed that natriuresis and diuresis produced by intracerebroventricular administration of clonidine is in part mediated by renal nerves.**

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Clonidine, an α2-agonist, is clinically used as an antihypertensive drug. The antihypertensive action of clonidine has been proposed to be mediated via central inhibition of sympathetic outflow (1). In addition, either intravenous (2) or intracerebroventricular (3) administration of clonidine causes diuresis and natriuresis. Previous studies have suggested that clonidine can alter sodium and water excretion by inhibiting renal nerve activity, by direct actions on the kidney, and by inhibiting vasopressin secretion (2, 4). Presently, the relative contribution of each of these mechanisms to the natriuretic response to intracerebroventricular administration of clonidine is not clear. Although the cause of the diuresis has mainly been attributed in part to inhibition of vasopressin, the cause for natriuresis remains to be elucidated (1). Renal nerves are known to be involved in the regulation of sodium excretion (4). It may be that clonidine produces natriuresis by producing renal sympatho-inhibition.

The purposes of the present experiments were: (i) to determine the involvement of renal nerves in the natriuresis and diuresis produced by central administration of clonidine, and (ii) to determine whether the natriuresis and diuresis produced by central administration of clonidine were in part due to a peripheral action of clonidine (that diffused out from the intracerebroventricular fluid into the peripheral circulation) directly on the kidney.

Methods

Male Sprague-Dawley rats (200–250 g) were anesthetized with Inactin (0.1 g/kg, ip). Body temperature

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was maintained between 36°C 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 polyethylene tubing and connected to a pressure transducer (Gould P23ID) for the continuous recording of arterial pressure. The left femoral vein was cannulated with PE-50 tubing, and a constant infusion (20 μl/min) of isotonic saline was started.

Renal Denervation and Ureteral Cannulation. The kidneys were exposed through an abdominal incision, and left renal denervation was performed by stripping the sheath and adventitia from the exposed left renal artery and vein. To destroy any remaining nerve fibers, the renal vessels were painted with 95% ethanol. Previously, this technique has been shown to decrease renal norepinephrine concentration <5% (5). In addition the urine output from the denervated kidney was consistently greater than the contralateral intact kidney in the present study.

Subsequently, both ureters were cannulated with PE-10 tubing. Surgery was completed within 60 min, and an additional 15-min stabilization period was allowed before the start of the first urine collection.

Placement of Cannula into the Intracerebroventricle. Rats were placed in a stereotaxic apparatus (Davis Kopf Instruments, Tujanga, CA). The coordinates for the left cerebral ventricle were determined from the atlas by Pellegrino *et al.* (6) (AP 1.0, L 1.2, DV 3.5 in relation to bregma). The cannula was lowered through a small trephined hole in the skull. The injection cannula was constructed from 20-gauge steel tubing. The cannula was then attached via polyethylene PE-50 tubing to a syringe filled with clonidine solution for infusion. Clonidine solution was made by dissolving the hydrochloride salt of clonidine in physiologic saline. The injectate was titrated to pH 7.3. The infusion rate of the pump was set at 2 μl/min. A saline vehicle infusion was also done to test for nonspecific effects of intracerebroventricular infusion.

Before sacrifice, injection sites were marked in each rat by including Evans blue dye in the infusate. The brain was subsequently removed and placed in a jar of 10% neutral formalin. Later the brain was sectioned to histologically verify the injection site. Only data from rats that showed spread of the dye within the cerebroventricular system were subsequently used for analysis. The 15-min period before infusion of drugs was considered the control period and the third collection period during clonidine infusion was reported as the clonidine infusion period (by the third collection, the response to clonidine infusion had stabilized). Data from these collection periods were analyzed and are reported in this manuscript.

Protocol. *Experiment 1: Renal responses to central administration of clonidine at different doses.* The natriuretic and diuretic effects of intracerebroventricular administration of clonidine were characterized in this experiment. Animals were divided into three groups. The first group of animals was infused with 8 μg/kg/min, the second group was infused with 2 μg/kg/min, and the third group was infused with vehicle to serve as time controls for the other two groups. Urine was collected in preweighed tubes, and urine volume was measured gravimetrically. After two 15-min control collection periods, urine was collected during three consecutive 15-min periods during which clonidine was infused into the intracerebroventricle. Subsequently, sodium (ion-selective electrode; Beckman Ion Analyzer) and osmolality (5100 vapor pressure osmometer; Wescor) of each of the urine samples were analyzed.

Experiment 2: Renal responses to central administration of clonidine (2 μg/kg/min) with simultaneous peripheral (intravenous) administration of yohimbine (specific α2-antagonist). The purpose of this experiment was to determine the peripheral component of the renal responses to central administration of clonidine. The protocol of this experiment was similar to the one described above, with the exception that an infusion of yohimbine (8 μg/kg/min, iv), an α2-antagonist, was started 15 min before the clonidine was administered into the intracerebroventricle. Yohimbine is a competitive antagonist and so we used four times the dose of the clonidine to block the effects of clonidine (7, 8).

Experiment 3: Renal responses to central administration of clonidine (0.2 μg/kg/min) at a dose that does not produce natriuresis and diuresis when administered peripherally. The purpose of this experiment was similar to the one for Experiment 2, but the approach was to use a 10-fold *lower* dose of clonidine (0.2 μg/kg/min) to minimize the amount of clonidine diffusion out of the intracerebroventricular space. In addition, a second group of rats was infused with the same lower dose of clonidine intravenously to demonstrate that a comparable dose peripherally does not produce the responses observed with the central low dose.

Experiment 4: Changes in glomerular filtration rate and filtration fraction of sodium to central administration of clonidine (2 μg/kg/min). The purpose of this experiment was to measure the glomerular filtration rate and filtration fraction of sodium to determine whether the changes observed in sodium excretion were due to changes in hemodynamic parameters or tubular actions. The protocol of this experiment was similar to the one described above (Experiment 1), with the exception that a bolus dose of inulin (10% inulin in 0.7 ml) followed by an infusion of inulin (2% at 20 μl/min) was started before the control collection. Blood samples (200 μl) were taken at midpoints of control period and

the third clonidine infusion period. Inulin concentrations were measured in the blood samples and the corresponding urine samples using the anthrone method (9). The clearance of inulin was used as an estimate of the glomerular filtration rate (GFR). Sodium excretion was calculated as net (sodium concentration in urine × urine flow) and as percentage of filtered sodium (fractional excretion).

Data Analysis. All data are expressed as mean value ± SE. The blood pressure data were subjected to analysis of variance for repeated measures followed by Duncan's multiple range test to assess the difference between the various groups and the control saline group (10, 11). Significant differences among urine flow, sodium excretion, and urine osmolality before and during infusion of clonidine were evaluated using the Student's t test for dependent means (10). A probability ($P < 0.05$) was considered statistically significant.

Results

Effect of Clonidine on the Mean Systemic Blood Pressure. Mean arterial pressure responses to various doses of intracerebroventricular or intravenous clonidine in the presence and absence of yohimbine are presented in Table I. Control arterial pressures (Time 0) were similar in all groups (no significant difference by multiple range test), and there was no change in pressure over the course of the experiments in the time control rats given saline alone. Analysis of variance for repeated measures with multiple independent groups showed significant effect of time. Clonidine infusion at a dose of 8 μg/kg/min produced a rise in blood pressure over time for the duration of the experiment (with a mean increase of 23 mm Hg by the end of the infusion period). A dose of 2 μg/kg/min produced a significant decrease in arterial pressure regardless of the presence of peripheral infusion of yohimbine compared with the saline-infused group (with a average decrease in pressure of 18–20 mm Hg by the end of the infusion period). The lowest dose of clonidine (0.2 μg/kg/min, intracerebroventricular as well as intravenous) also produced a statistically significant decrease in arterial pressure over time (a mean decrease of 25 mm Hg by the end of the infusion period); however, the pressure at the end of the infusion period was not statistically different from that of the saline-infused group.

Experiment 1: Renal Responses to Central Administration of Clonidine at Different Doses. Baseline urine flow and sodium excretion were elevated after renal denervation (Fig. 1). Central administration of clonidine (both doses 2 and 8 μg/kg/min) produced a significant increase in urine flow and sodium excretion from intact kidneys. Although 8 μg/kg/min of clonidine produced a significant increase in diuresis and natriuresis from the denervated kidneys, a 2-μg/kg/min dose of clonidine failed to produce a natriuresis from the denervated kidneys (Fig. 1B). A dose of 2 μg/kg/min did produce a diuresis from the denervated kidneys (Fig. 1A). As would be expected, the urinary osmolality decreased in response to central administration of clonidine (Table II), similar to previous reports in the literature (3).

There was an increase in sodium excretion from the intact kidney but a decrease from the denervated kidney at a dose of 2 μg/kg/min of clonidine (Fig. 1B). Although the changes (Δ) are not indicated on Figure 1, a dose of 8 μg/kg/min of clonidine produced a greater change (increase) in sodium excretion from the intact kidney compared with the denervated kidney.

The saline control (time control and Time 0 dose of clonidine) group demonstrated that there was no significant change in urine flow or sodium excretion over time.

Experiment 2: Renal Responses to Central Administration of Clonidine (2 μg/kg/min) with Simultaneous Peripheral (intravenous) Administration of Yohimbine (specific α2-receptor antagonist). Again renal denervation produced a greater natriuresis and diuresis from the denervated kidney compared with the intact kidney (Fig. 2). Peripheral administration of yohimbine (8 μg/kg/min, iv) did not produce any significant changes in urine flow or sodium excretion (Table

Table I. Mean Arterial Pressure in Different Groups of Rats[a]

Doses of clonidine (μg/kg/min)	Before clonidine	Clonidine		
	0 min (mm Hg)	15 min (mm Hg)	30 min (mm Hg)	45 min (mm Hg)
Saline (icv) ($n = 5$)	108 ± 5	113 ± 5	108 ± 5	110 ± 5
8.0 (icv) ($n = 5$)	95 ± 5	116 ± 10	118 ± 10	118 ± 8
2.0 (icv) ($n = 7$)	110 ± 3	107 ± 5	106 ± 3	89 ± 3[b]
0.2 (icv) ($n = 6$)	118 ± 5	113 ± 6	104 ± 6	93 ± 7
0.2 (iv) ($n = 6$)	119 ± 9	116 ± 6	104 ± 8	94 ± 5
2.0 (icv) + yohimbine (8 μg/kg/min iv) ($n = 7$)	107 ±7	104 ± 6	94 ± 7	89 ± 6[b]

[a] Values represent mean ± SE.
[b] $P < 0.05$ versus saline control.

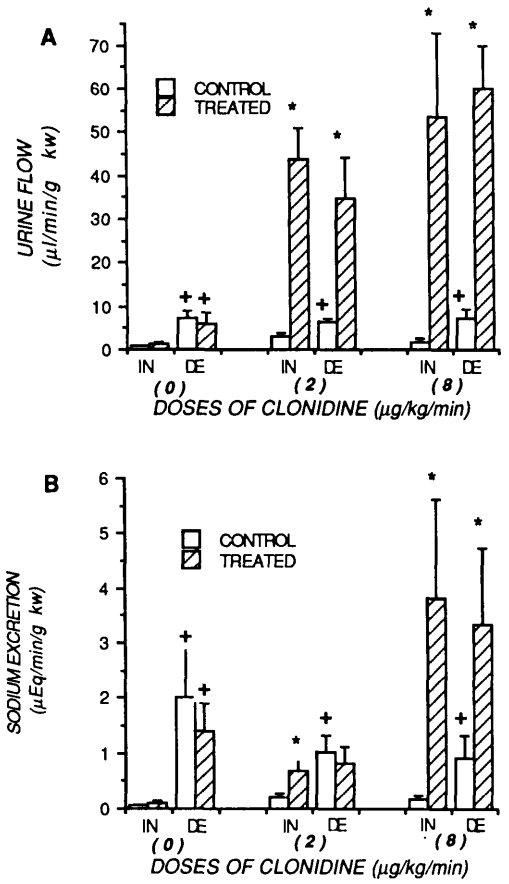

Figure 1. (A) Urine flow and (B) sodium excretion from intact (IN) and denervated (DE) kidneys before (control) and during intracerebroventricular administration of saline or clonidine (0, 2.0, and 8.0 μg/kg/min, treated). Values represent mean ± SE (n = 5–7). Asterisk indicates $P < 0.05$ versus control. Plus sign (+) indicates $P < 0.05$ versus IN at the same dose. kw, Kidney weight.

III). However, central administration of clonidine (2 μg/kg/min) in the presence of yohimbine (8 μg/kg/min, iv) produced a significant increase in urine flow from both intact and denervated kidneys and an increase in sodium excretion from the intact kidney (Fig. 2).

Experiment 3: Renal Responses to Central Administration of Clonidine (0.2 μg/kg/min) at a Dose that Does Not Produce Natriuresis and Diuresis when Administered Peripherally. Renal denervation once again produced a greater natriuresis and diuresis from the denervated kidney compared with the intact kidney (Fig. 3). Central administration of clonidine at a dose of 0.2 μg/kg/min produced a significant increase in natriuresis and diuresis from the intact kidney and a significant increase in diuresis from the denervated kidney (Fig. 3). At this same low dose, there was no natriuresis or diuresis from either kidneys when clonidine was administered peripherally (Fig. 3).

Experiment 4: Changes in GFR and Filtration Fraction of Sodium to Central Administration of Clonidine (2 μg/kg/min). Renal denervation once again produced a greater natriuresis and diuresis from the denervated kidney compared with the intact kidney (Table IV). Central administration of clonidine at a dose of 2 μg/kg/min produced a significant increase in natriuresis and diuresis from the intact kidney and a significant increase in diuresis from the denervated kidney, as observed above. There was no significant difference in GFR between the intact and denervated kidneys before or after the central administration of clonidine (Table IV). However, there was a significantly greater increase in fractional excretion of sodium from the intact kidneys, but not the denervated kidneys, after central administration of clonidine.

Discussion

This study demonstrates that centrally administered intracerebroventricular clonidine produces diuresis and natriuresis that are partly mediated by renal nerves. Particularly, the natriuresis seems to be mediated by renal sympatho-inhibition. When peripheral α2-receptors are blocked by yohimbine, central administration of clonidine still produces diuresis and natriuresis from the intact kidney, although the response was reduced. In addition, central administration intracerebroventricular of a low dose of clonidine produces diuresis and natriuresis from intact kidneys, even though a similar dose given peripherally intravenous does not. Taken together, these studies suggest that clonidine produces natriuresis via renal nerves by its action on the central nervous system.

The diuretic action of clonidine administration have been explained previously by inhibition of vasopressin release (2). Pettinger *et al.* (12) have also suggested a possible inhibitory action of clonidine on the actions of vasopressin at the kidney. Therefore, the diuretic responses in the present study are probably

Table II. Urine Osmolality before and after Central Administration of Clonidine[a]

Doses of clonidine (icv)	Before clonidine		After clonidine	
	Intact	Denervated	Intact	Denervated
8.0 μg/kg/min (n = 5)	1301 ± 141	1369 ± 200	488 ± 304[b]	612 ± 373[b]
2 μg/kg/min (n = 7)	1812 ± 242	1394 ± 174	839 ± 246[b]	882 ± 177[b]

[a] Values represent mean ± SE.
[b] $P < 0.05$ versus Before clonidine.

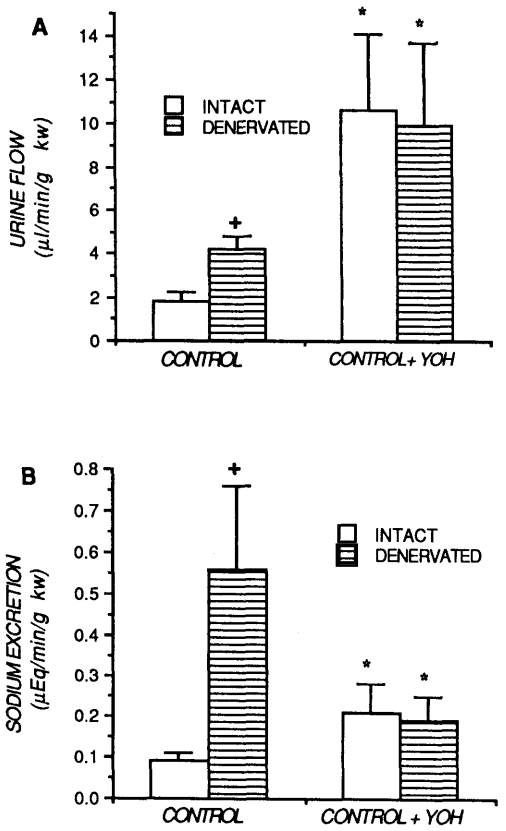

Figure 2. (A) Urine flow and (B) sodium excretion from intact and denervated kidneys before (control) and during intracerebroventricular administration of clonidine (2.0 μg/kg/min) with an infusion of yohimbine (8 μg/kg/min) already present (control + YOM). Values represent mean ± SE (n = 7). Asterisk indicates $P < 0.05$ versus control. kw, Kidney weight.

related in part to the inhibition of release and actions of vasopressin (2, 12). However, the question regarding the reason for the natriuretic response to administration of clonidine remained unanswered by these studies and is addressed in this study.

The actions of centrally administered clonidine are known to be sympatho-inhibition via action on the central nervous system (1). This study shows that there may be renal sympatho-inhibition that leads to natriuresis. Koepke and DiBona (13) and Koepke *et al.* (14) have shown that renal nerve activity decreases when clonidine is administered centrally. Renal nerves are known to have three functions in the kidney (i) vasoconstriction, (ii) renin release, and (iii) sodium reabsorption in the proximal tubules; all of them lead to retention of sodium (4). With central administration of clonidine (2 or 0.2 μg/kg/min), the renal sympatho-inhibition may lead to the increased natriuresis observed in the intact kidney; at the same time, there was an absence of an increase in natriuresis from the denervated kidney. It is of interest to note that at the higher dose of clonidine (8 μg/kg/min), there was an increase in sodium excretion from the denervated kidney as well. This may be because the higher arterial pressure (an increase in pressure at this dose compared with a decrease in pressure at the lower doses) in this group may have increased sodium excretion in the denervated kidney through pressure-natriuresis or a possible nonspecific action of clonidine on some other site(s) to produce this effect.

The specific intrarenal site of action of clonidine intracerebroventricular as is expressed via the renal nerves remains unsolved. It is an important and a complex question that needs further study. Nevertheless, it is of interest to note that in the study by Roman *et al.* (2), the authors did not observe any changes in GFR or renal plasma flow after administration of clonidine. We have observed similar results in a group of rats infused with clonidine centrally (Experiment 4). Roman *et al.* (2) concluded that the actions of clonidine were not dependent upon the changes in hemodynamic parameters. After measurements of distal delivery of sodium, Roman *et al.* (2) suggested that "the tubular site at which clonidine inhibits reabsorption of sodium appears to be in nephron segments proximal to the diluting nephron segments," i.e., in the proximal tubule or the descending or ascending thin limb of Henle. Our data show that there is reduced tubular sodium reabsorption in the intact kidney, but not the denervated kidney, during central administration of clonidine. It is possible that the natriuretic response to intracerebroventricularly administered clonidine may be expressed via the actions of renal nerves on the proximal tubules and/or the descending or ascending thin limb of Henle. The specific site of action within the nephron remains to be examined.

Centrally administered clonidine may be having a peripheral action because clonidine is known to diffuse

Table III. Urine Flow and Sodium Excretion before and after Intravenous Administration of 8 μg/kg/min of Yohimbine[a]

	Urine flow (μl/min/g kidney wt)		Sodium excretion (μEq/min/g kidney wt)	
	Intact	Denervated	Intact	Denervated
Before yohimbine	2.0 ± 0.6	4.7 ± 0.9	0.16 ± 0.05	2.19 ± 1.37
After yohimbine	2.5 ± 0.7	4.6 ± 0.6	0.17 ± 0.06	2.22 ± 1.41

[a] Values represent mean ± SE (n = 7). No significant differences between before and after yohimbine.

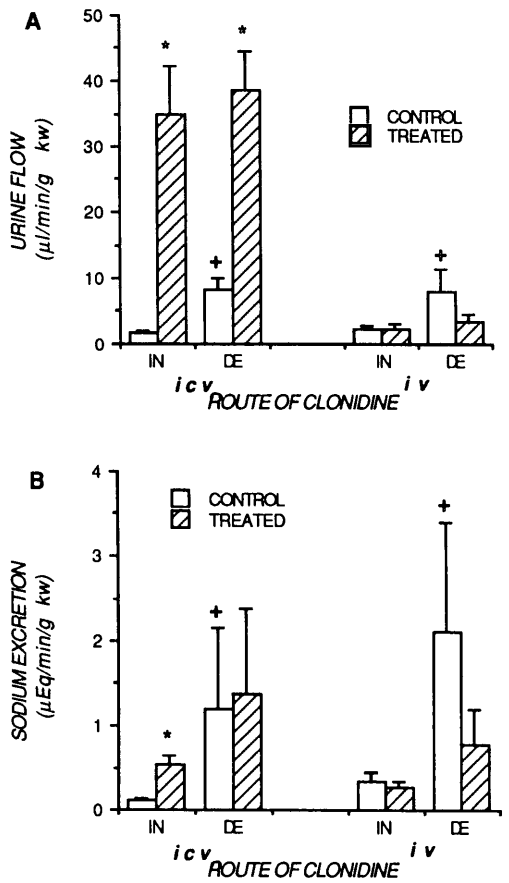

Figure 3. (A) Urine flow and (B) sodium excretion from intact (IN) and denervated (DE) kidneys before (control) and in response to intracerebroventricular or intravenous administration of clonidine (0.2 μg/kg/min, treated). Values represent mean ± SE ($n = 6$). Asterisk indicates $P < 0.05$ versus control. Plus sign (+) indicates $P < 0.05$ versus IN by the same route. kw, Kidney weight.

out of intracerebroventricular space via sites with a weak blood-brain barrier, e.g., subfornical organs. In an attempt to exclude this possibility, two experiments were performed: (i) yohimbine was administered intravenously to block the peripheral actions of clonidine, and (ii) a dose 10-fold smaller administered peripherally did not produce any of the natriuretic and diuretic effects observed when this dose was given centrally. Both these experiments confirmed a sympatho-inhibitory component involved in the natriuresis observed during central administration of clonidine. Since it is possible that yohimbine crossed the blood-brain barrier and inhibited the central action of clonidine, the results obtained with yohimbine may be deemed inconclusive. Nevertheless, it is of interest that the peripherally administered yohimbine did not affect the hypotension produced by central administration of clonidine (Table I). It could be deduced that yohimbine had its major inhibitory effect peripherally, since it did not block the hypotensive effect of centrally administered clonidine. Alternatively, it could be argued that although peripheral yohimbine did not alter the hypotension produced by central administration of clonidine, yohimbine may still be inhibiting the renal nerve activity centrally. This may also explain the slight attenuation of the natriuretic response to clonidine from the intact kidney after the administration of yohimbine (Fig. 2).

Another possible mechanism that may be involved in the natriuresis by the action of clonidine could be the release of atrial natriuretic factor (15). This possibility can be ruled out because of two reasons: (i) the denervated kidney that was exposed to the same hormonal influence as the intact kidney did not show natriuresis to central administration of clonidine, and (ii) some preliminary atrial natriuretic factor measurements in our laboratory did not show an increase in atrial natriuretic factor level after central administration of clonidine (unpublished results).

Centrally administered clonidine may leak out of the blood-brain barrier and have a peripheral action directly on the kidney (12). Pettinger *et al.* (12) have advanced the view that activation of $\alpha 2$ receptors in the kidney opposes the action of vasopressin in the kidney and produces diuresis and natriuresis. The present study would not support this view, at least at the low dose of clonidine (0.2 μg/kg/min), which produced diuresis and natriuresis upon central administration but failed to alter the urine flow and sodium excretion upon intravenous administration. These data would suggest that clonidine can have its diuretic and natriuretic effects by its action centrally at fairly low doses. At the higher doses (2–8 μg/kg/min), clonidine did produce a

Table IV. Urine Flow, Sodium Excretion, Glomerular Filtration, Renal Plasma Flow, and Fractional Excretion of Sodium before and after Central Administration of 2 μg/kg/min of Clonidine[a]

	Before clonidine		After clonidine	
	Intact	Denervated	Intact	Denervated
Urine flow (μl/min/g kidney wt)	3.3 ± 0.8	8.4 ± 0.9	17.4 ± 3.7[b]	16.3 ± 4.5[b]
Sodium excretion (μEq/min/g kidney wt)	0.15 ± 0.02	0.98 ± 0.26	0.74 ± 0.07[b]	0.65 ± 0.27
GFR (ml/min/g kidney wt)	0.78 ± 0.11	0.92 ± 0.08	0.91 ± 0.25	1.09 ± 0.22
Fractional Na^+ excretion (%)	0.21 ± 0.01	0.76 ± 0.34	0.40 ± 0.01[b]	0.56 ± 0.32

[a] Values represent mean ± SE ($n = 5$).
[b] $P < 0.05$ versus Before clonidine.

diuresis from the denervated kidneys, which indicates a peripheral action directly on the kidney of clonidine as proposed by Pettinger *et al.* (12), pressure-natriuresis, or a combination of these effects.

The efficacy of clonidine as a potent antihypertensive agent has been attributed to its central action to reduce sympathetic tone (1). The results from this study suggest that clonidine has a powerful diuretic and natriuretic effect that appears to be produced by a renal sympatho-inhibitory effect. Combining these results with the hypothesis proposed by Guyton *et al.* (16) that kidney function is the long-term controller of arterial pressure, would imply that clonidine has its antihypertensive action by its indirect action on the kidney via renal sympatho-inhibition preventing sodium retention, thus resetting kidney function, rather than via the general sympatho-inhibition to all vascular beds per se. Second, the fact that clonidine also produces huge diuresis and natriuresis that are due to its direct action on the kidney implies that at large doses, the antihypertensive action of clonidine may be due to this direct action of clonidine on the kidney.

It should be noted that the preparation used in this study, i.e., unilaterally renal denervated rats, may have more renal nerve activity in the innervated kidney because of the contralateral denervation and renorenal reflex (17) and increased renal nerve activity in Inactin-anesthetized rats (18). It may be argued that it is easier to observe an inhibition of renal nerve activity under such circumstances (i.e., unilateral denervation). Nevertheless, this does not negate the renal sympatho-inhibition and subsequent natriuresis from the intact kidney by clonidine.

In summary, this study demonstrates that centrally administered clonidine produces diuresis and natriuresis that is mediated partly by renal sympatho-inhibition. Second, the natriuresis produced by central administration of clonidine appears to be due totally to renal sympatho-inhibition. It is interesting to speculate that the majority of the antihypertensive action of clonidine may be via its indirect action on the kidney via renal sympatho-inhibition over a long term.

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