Diuretic Treatment Alters Clonidine Suppression of Plasma Norepinephrine

THOMAS P. HUI, LAWRENCE R. KRAKOFF, CATHERINE FELTON, AND KAREN YEAGER

SUMMARY The effect of short-term diuretic treatment on the action of clonidine was evaluated in eight subjects with mild, uncomplicated hypertension. A single oral dose of clonidine (0.3 mg) was given before and after 1 week of therapy with hydrochlorothiazide, 50 mg, and amiloride, 5 mg, taken daily. Changes in mean arterial pressure, heart rate, plasma norepinephrine and epinephrine levels, and plasma renin activity were assessed. Diuretic treatment caused a significant weight loss, increased plasma renin activity, and reduced serum potassium concentration but did not significantly alter the absolute reduction in mean arterial pressure caused by clonidine. Absolute clonidine-induced reduction in plasma renin activity after diuretic treatment was three times greater than before treatment, although percent changes were similar. Before diuretic therapy, clonidine significantly reduced the level of norepinephrine (absolute and percent change). After diuretic treatment, clonidine failed to suppress norepinephrine, and the difference from prediuretic changes was significant. The level of epinephrine was not altered significantly either by diuretic treatment or clonidine. These results indicate that diuretic therapy alters the clonidine-activated mechanism for reduction of arterial pressure through a shift from overall suppression of sympathetic tone to pathways that are more restricted to renal tone. This shift may be due to changes in fluid or electrolyte balance that alter the action of $\alpha_2$-adrenergic receptor-mediated pathways. Use of the clonidine suppression test for the diagnosis of pheochromocytoma may give false-positive results in diuretic-treated patients.

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KEY WORDS • $\alpha_2$-adrenergic receptor agonist • catecholamines • plasma renin activity

CLONIDINE, an $\alpha_2$-adrenergic receptor agonist, is one of several antihypertensive drugs whose major site of action occurs within the central nervous system. Its hypotensive effect is due to reduction of peripheral sympathetic outflow as a result of central stimulation of $\alpha_2$-adrenergic receptors. Diuretics are also effective antihypertensive agents that reduce pressure by depleting extracellular fluid volume and electrolytes. Diuretic administration and low salt intake tend to increase sympathetic tone, as reflected by elevations of plasma catecholamine concentrations.

Combining clonidine with a diuretic for the treatment of hypertension leads to a greater reduction in arterial pressure than when either drug is given alone. To account for this effect, we postulated that clonidine causes a greater inhibition of sympathetic tone by its action on central $\alpha_2$-adrenergic receptors in diuretic-treated patients. To test this hypothesis, clonidine suppression of plasma catecholamine concentration and plasma renin activity (PRA) was evaluated before and after 1 week of diuretic treatment in a group of subjects with mild, uncomplicated hypertension.

Subjects and Methods

Subjects were selected from an outpatient hypertension clinic population. Previous evaluation demonstrated that none had secondary hypertension, diabetes, or clinically significant cardiac, renal, or neurological disease. Eight subjects (5 men and 3 women) with mild essential hypertension (diastolic pressure, 90-104 mm Hg) were studied. Mean age (± SE) was 50 ± 5 years. Four subjects were previously untreated; the other four had been treated with a diuretic preparation that was discontinued 2 weeks before the study began. The research protocol was approved by the Mount Sinai Hospital Committee on Human Research; all subjects gave written informed consent for their participation.

A clonidine suppression test, given in a modified...
form as described in the next paragraph, was performed in each subject on 2 separate days, before and after 1 week of diuretic therapy. The diuretic employed was a combination of hydrochlorothiazide, 50 mg, and amiloride, 5 mg, taken once daily. This preparation was chosen to prevent the potassium loss and hypokalemia that might alter sympathetic function. Subjects were advised not to change their usual diet. No potassium supplements or additional medications were permitted. The effects of diuretic treatment on weight, serum electrolyte levels, and urine sodium and potassium excretion were evaluated. Twelve-hour urine samples were collected from 1900 on the evening before to 0700 on the day of each clonidine suppression study.

Studies were conducted in the morning beginning at 0800 on subjects who had been fasting since the previous evening. On arrival at the study area, subjects were weighed and then placed in the supine position. A catheter then was inserted in a forearm vein to obtain blood samples without additional puncture. Systemic arterial blood pressure was measured by an automated device (Infrasonde D-4000, Puritan-Bennett, Los Angeles, CA, USA), and heart rate was determined by electrocardiogram at 10-minute intervals. Subjects then were observed for 60 minutes, after which blood samples were obtained for serum chemistry values, PRA, and plasma catecholamine concentration. Subjects then received 0.3 mg of clonidine by mouth with 1 dl of water. Additional blood samples were obtained with subjects in the supine position at 1 and 2 hours after clonidine administration. Subjects then stood upright for 10 minutes, and the final blood samples were obtained. Plasma catecholamine determinations were made at the end of the control period, 1 and 2 hours after clonidine administration with subjects in the supine position, and after 10 minutes of upright posture. The PRA was determined at the end of the control period and 2 hours after clonidine administration.

Serum and urine chemistry values were determined by automated standard clinical methods. The PRA and plasma catecholamine concentrations were determined by radioimmunoassay and radioenzymatic measurement, respectively.

Single comparisons were assessed by the t test as appropriate for paired or unpaired variates. For multiple measurements, the repeated-measures analysis of variance procedure was employed as recommended for studies of this type. The BMDP programs of the CUNY Computer Center were employed for this purpose. Significance was accepted at a p level of 0.05. Results are presented as means ± SE.

Results

The enrolled subjects completed each phase of the study, so that the results presented summarize all observations on all subjects.

The effect of diuretic administration on body weight, several serum biochemical indices, and overnight urinary excretion of sodium and potassium are given in Table 1. Significant reductions in weight and serum sodium, potassium, and chloride levels were observed. Twelve-hour urine potassium excretion was increased significantly, but sodium excretion was unchanged.

Determination of mean arterial pressure and heart rate during the various phases of the clonidine suppression studies before and after the week of diuretic administration are shown in Figure 1. Both absolute values for all phases and percent change with subjects in the supine position are shown. Statistical analysis by analysis of variance indicated that mean arterial pressure was reduced significantly after diuretic administration to supine subjects (p = 0.02; see Figure 1, top panel) and that the effect of clonidine was highly significant (p < 0.01). No significant interaction effect occurred (p > 0.3); in other words, diuretic administration did not alter the overall magnitude of the effect of clonidine on arterial pressure.

The bottom panel of Figure 1 shows percent changes in mean arterial pressure caused by clonidine adminis-

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**Table 1. Effect of Diuretic Treatment on Weight, Serum Chemistry Values and 12-Hour Sodium and Potassium Excretion**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-diuretic</th>
<th>Post-diuretic</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>75 ± 3.0</td>
<td>74.0 ± 3.3</td>
<td>-1.8 ± 0.5*</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺ (mEq/L)</td>
<td>140 ± 1</td>
<td>136 ± 1</td>
<td>-4 ± 1*</td>
</tr>
<tr>
<td>K⁺ (mEq/L)</td>
<td>44 ± 0.1</td>
<td>38 ± 0.1</td>
<td>-6 ± 0.1*</td>
</tr>
<tr>
<td>Cl⁻ (mEq/L)</td>
<td>107 ± 1</td>
<td>100 ± 2</td>
<td>-7 ± 2*</td>
</tr>
<tr>
<td>CO₂ (mEq/L)</td>
<td>24 ± 1</td>
<td>25 ± 1</td>
<td>+ 1 ± 1</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>14 ± 2</td>
<td>17 ± 2</td>
<td>+ 3 ± 2</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>53 ± 0.4</td>
<td>64 ± 0.6</td>
<td>+ 11 ± 0.5</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>108 ± 7</td>
<td>119 ± 4</td>
<td>+ 11 ± 5</td>
</tr>
<tr>
<td>Urine (12-hour excretion)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺ (mEq/L)</td>
<td>96 ± 20</td>
<td>98 ± 20</td>
<td>+ 2 ± 10</td>
</tr>
<tr>
<td>K⁺ (mEq/L)</td>
<td>24 ± 3</td>
<td>43 ± 6</td>
<td>+ 19 ± 7†</td>
</tr>
</tbody>
</table>

Values are means ± SE

*p < 0.01, †p < 0.05, for change

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**Figure 1. Effect of clonidine on mean arterial pressure (MAP) and heart rate before (PRE) and after diuretic treatment. Statistical analysis is given in the text.**
tration to subjects in the supine position. The overall percent change was highly significant \((p < 0.01)\) but did not differ between prediuretic and postdiuretic studies \((p > 0.5)\). As indicated by the slopes of the two lines, a highly significant percent change in mean arterial pressure occurred between 1 and 2 hours after clonidine administration \((p < 0.01)\).

Heart rate (absolute values) was not significantly affected by diuretic administration \((p = 0.5; \text{see Figure 1})\); however, significant changes were observed after clonidine administration and after subjects stood for 10 minutes \((p < 0.001)\). No significant interaction between diuretic administration and clonidine was detected \((p = 0.1)\). Figure 1 displays percent change in heart rate for subjects in the supine position. Clonidine caused a significant percent reduction in heart rate \((p = 0.01)\), but neither a diuretic effect \((p > 0.2)\) nor time dependency \((p > 0.2)\) was noted.

Measurements of PRA during the two clonidine suppression studies are shown in Figure 2. Diuretic treatment produced an increase in PRA of \(8.0 \pm 2.4 \text{ ng/ml/hr} (p < 0.02)\) before clonidine administration. A highly significant effect of clonidine on PRA was observed before and after diuretic treatment \((p < 0.01)\). The interaction effect was not significant \((p > 0.1)\); however, the absolute clonidine-induced reduction in PRA after diuretic administration \((-2.7 \pm 1.0 \text{ ng/ml/hr}; p < 0.05)\) was three times greater than that observed before the diuretic was given \((-0.8 \pm 0.3 \text{ ng/ml/hr}; p < 0.05)\).

Measurements of plasma norepinephrine concentration during all phases of the studies are shown in Figure 3. Before diuretic administration, clonidine significantly reduced subjects' plasma norepinephrine concentration after 1 hour \((-250 \pm 80 \text{ pg/ml}; p < 0.02)\) and 2 hours \((-295 \pm 95 \text{ pg/ml}; p < 0.02)\) in the supine position. Standing increased plasma norepinephrine concentration in six of eight subjects, but the mean change was not statistically significant \((-357 \pm 295 \text{ pg/ml}; p > 0.1)\). After diuretic treatment, the effect of clonidine on the plasma norepinephrine concentration of subjects in the supine position was not significant at either 1 hour \((-178 \pm 107 \text{ pg/ml}; p > 0.1)\) or 2 hours \((-47 \pm 89 \text{ pg/ml}; p > 0.2)\). A highly significant increase occurred when the subjects assumed an upright posture \((-582 \pm 140 \text{ pg/ml}; p < 0.01)\). Clonidine had no significant effect on plasma epinephrine concentration either before or after diuretic treatment.

Comparison of the effects of clonidine on plasma epinephrine, plasma norepinephrine, and PRA are given in Figure 4, where changes occurring when the subjects were supine are shown as percent deviation from preclonidine concentrations. It is apparent that the only significant effect of diuretic administration was the change in plasma norepinephrine concentration.

**Discussion**

This study was undertaken to evaluate the effect of short-term diuretic administration on the response to clonidine, as used to distinguish between essential hypertension and pheochromocytoma.\(^1\) We predicted that diuretic treatment would cause a hyperadrenergic state and that clonidine would have a greater effect in lowering both arterial pressure and plasma norepinephrine concentration after volume depletion.
subjects demonstrated changes typical of diuretic use: statistically significant weight reduction and increased PRA. The changes in serum electrolyte concentration and in urine potassium excretion were also consistent with the known action of the diuretic combination used. A small but significant reduction in serum potassium occurred; however, the mean serum potassium concentration after 1 week of treatment with 50 mg of hydrochlorothiazide and 5 mg of amiloride remained above the lower limit of normal, 3.5 mEq/L. Diuretic treatment markedly increased control PRA but did not significantly increase control plasma norepinephrine concentration. The latter result differs from the report of Lake et al., who, in a larger series, found that hydrochlorothiazide alone significantly increased supine plasma norepinephrine concentration. The contrast between our results and theirs may be due to differences in sample size or to the diuretic agents studied.

We found that clonidine’s effect on arterial pressure was not augmented by diuretic treatment but instead appears to be additive, so that the percent change in arterial pressure caused by clonidine was not different before or after diuretic treatment, as shown in Figure 1. The effects of clonidine administration on PRA and plasma norepinephrine concentration before diuretic treatment were entirely consistent with several previous studies. Goldstein et al. found no correlation between the variation in urine sodium excretion over a fivefold, but normal, range and the degree of clonidine-induced suppression of plasma norepinephrine. No subjects were taking diuretics and PRA was not reported. We found that clonidine administration following 1 week of diuretic treatment reduced arterial pressure and PRA in a manner similar to that observed before the diuretic was given. In contrast, clonidine’s effect on plasma norepinephrine concentration was altered by diuretic treatment in that the significant reduction noted with the subjects in the supine position no longer occurred and the response to subjects’ upright posture was unimpaired.

The antihypertensive mechanism of clonidine is thought to be due to its action within the central nervous system as an α₂-agonist in reducing peripheral sympathetic tone. However, reduction of norepinephrine release by presynaptic α₂-adrenergic receptor activation of peripheral adrenergic neurons may also participate in clonidine’s overall effect.

Recent studies suggest that the binding properties of central and peripheral α₂-agonists may be altered by changes in sodium ion concentration in vitro and by sodium balance in vivo. Such observations raise the possibility that the changes we observed in clonidine’s effect on plasma norepinephrine concentration in diuretic-treated subjects are due to alterations in the binding activities of α₂-adrenergic receptors as a result of the electrolyte alterations caused by diuretic treatment.

Other mechanisms also may account for the diminished action of clonidine in reducing overall sympathetic tone (as represented by plasma norepinephrine concentration) in volume-depleted subjects. Activation of low or high pressure baroreflexes by diuretic treatment may augment or recruit sympathetic tone by central pathways that are mediated by a variety of transmitters and thus bypass or alter central adrenergic pathways. The increased PRA we observed after diuretic treatment no doubt raises plasma angiotensin II concentration, which may have a central action that increases sympathetic tone by specific pathways.

Clonidine reduced arterial pressure in our subjects in the supine position both before and after diuretic treatment. This effect was not enhanced by the diuretic, but the pressures observed after both drugs represented an additive effect. Since diuretic treatment eliminated the clonidine-induced suppression of plasma norepinephrine, reduction in arterial pressure may have been due to the persisting effect of the α₂-agonist on the renin-angiotensin system. These observations suggest that diuretic treatment alters the action of clonidine (and possibly other α₂-agonists) from one that suppresses overall sympathetic tone in the normal or hypervolemic state to one that is more restricted to renal tone in the hypovolemic state. The studies of Wallin and Frisk-Holmberg support this hypothesis by indicating a highly variable effect of clonidine on sympathetic innervation to muscle, skin, and visceral structures.

In summary, short-term diuretic treatment diminished the suppressing effect of clonidine administration on plasma norepinephrine but did not alter the antihypertensive response to the α₂-agonist. The precise mechanisms accounting for this effect are not defined, nor is the duration of the effect established, as patients receiving diuretics for longer periods have not yet been studied.

One practical implication of our results applies to use of the clonidine suppression test in patients taking diuretics who are being evaluated for pheochromocytoma. Failure to observe a significant reduction in plasma norepinephrine concentration after clonidine administration may suggest a false diagnosis of a catecholamine-secreting tumor. It is our present practice to apply this diagnostic test only in patients withdrawn from all diuretic agents.

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