SUMMARY Participation of vasopressin and the renin-angiotensin system in the maintenance of systemic arterial pressure was evaluated in unanesthetized adrenalectomized rats. Adrenalectomized and sham-operated rats with implanted arterial and venous catheters were given 1% sodium chloride and 2.5% glucose as drinking fluid for 72 hours following adrenalectomy. Serum and urine samples were obtained for measurement of electrolyte and solute concentration. The pattern of serum electrolytes, serum osmolality, and renal excretion of electrolytes, solute, and water observed in the adrenalectomized rats was entirely consistent with previous observations in this model. Mean arterial pressure of unanesthetized unrestrained adrenalectomized rats was significantly lower than controls. In adrenalectomized rats, DPMeTyrAVP reduced mean arterial pressure 9 ± 1 mm Hg, p < 0.001; captopril then caused an additional reduction of 17 ± 2 mm Hg, p < 0.01. Neither antagonist altered arterial pressure in the control group. Our results indicate that vasopressin and the renin-angiotensin system play a compensatory pressor role in adrenal insufficiency, preventing a larger decrease of arterial pressure in this model of chronic hypotension.


**Key Words** • converting enzyme inhibitor • vasopressin antagonist • adrenal insufficiency • conscious rats

**Methods**

Female Sprague Dawley rats with an approximate weight of 250 g were placed in individual metabolic cages for a 3-day period of habituation after which they underwent either adrenalectomy or sham adrenalectomy through a lumbar incision. Surgery was carried out during anesthesia with ketamine (100 mg/kg ip). At this time a Tygon (Norton) catheter was inserted into the left carotid artery and advanced to the thoracic descending aorta. Two catheters were inserted in the left jugular vein and advanced to the superior vena cava, just proximal to the right atrium. All three catheters were exteriorized at the back of the neck, filled with heparin (100 U/ml), and capped with plugs. After recovery from anesthesia and surgery, animals were placed in their metabolic cages and were allowed free access to Purina Chow (0.4% NaCl) and 1% NaCl in 2.5% dextrose as drinking fluid for the next 72 hrs. Urine volume was measured daily and aliquots of urine were frozen for later measurement of electrolytes and osmolality.

Three days after surgery, blood pressure measurements were made in the unanesthetized unrestrained
animals. The arterial catheters were connected to a Physiograph DMP-4A recorder through a Statham P23Db transducer. Mean arterial pressure was obtained by electronic damping. After blood pressure stabilization, dPMeTyrAVP was injected i.v. (50 μg/kg) followed by captopril (1 mg/kg) approximately 20 minutes later. Each antagonist was administered through a separate venous catheter in a volume of vehicle no greater than 0.2 ml.

The dPMeTyrAVP was kept refrigerated in a 0.05 M glacial acetic acid, 0.5% chlorobutanol solution at 1 mg/ml. An aliquot was redissolved in 0.2 ml of saline immediately before injection. The dose employed in these experiments is equivalent to 100 times the calculated ID₅₀ for this compound. In preliminary experiments, it inhibited the pressor action of injected vasopressin (10 mU) by 95% for 4 hours. Captopril was prepared fresh as a 2 mg/ml solution in saline. In preliminary experiments, this dose, which is equivalent to 65 times its ID₅₀, inhibited the pressor response to injected angiotensin I (310 ng/kg) for at least 30 minutes.

Arterial blood samples were obtained at the end of the infusion study for measurement of serum osmolality, electrolytes and urea nitrogen. Additional samples for these measurements are included for those rats unsuitable for infusion because of occlusion of catheters by thrombosis. Urine and serum electrolytes were measured by flame photometry (Instrument Laboratories). Urine and serum osmolality were measured by freezing point depression (Fiske OM™ Osmometer) and serum urea nitrogen by use of the Dow Diagnostics Reagent in a Beckman Spectrophotometer.

Paired and unpaired Student's t tests were employed for statistical analysis. Results are expressed as mean ± SE.

Results

During the 3-day period after adrenalectomy or sham adrenalectomy, weight loss was similar in both groups (adrenalectomy 8 ± 5 g, controls 10 ± 6 g). As shown in table 1, hyponatremia, hyperkalemia, and a significant reduction in serum osmolality (6%) were found in adrenalectomized rats; serum urea nitrogen levels were not significantly different between the two groups. Urine potassium excretion was reduced by 49% in adrenalectomized rats (p < 0.05), but no significant difference was found for urinary sodium excretion. Despite the difference in serum osmolality, osmolar clearance and tubular reabsorption of water were similar in the two groups.

Measurements of mean arterial pressure in adrenalectomized and control rats and the effects of antagonists, dPMeTyrAVP and captopril, are shown in figure 1. Mean arterial pressure in adrenalectomized animals was 16% lower than in controls (p < 0.01) prior to pharmacologic interventions. Injections of dPMeTyrAVP reduced mean arterial pressure by 9 ± 1 mm Hg (p < 0.001) in the adrenalectomized group. Subsequent injection of captopril further reduced arterial pressure by 17 ± 2 mm Hg (p < 0.01). Thus, .

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<tr>
<th>Table 1. Serum and Urine Measurements in Adrenalectomized Rats</th>
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<td>Serum Na (mEq/liter)</td>
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<td>Serum Osm (mOsm/liter)</td>
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<td>Serum urea nitrogen (mg/dl)</td>
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<td>C₀₂OM (ml/hr)</td>
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Data are given as means ± SEM. Parentheses indicate size of each group.

The p values are for the unpaired comparisons between control and adrenalectomized groups.

Urinary excretion of sodium and potassium is given as the total for the three days of the study. Osmolar clearance and tubular reabsorption of water were calculated from observations of the third 24-hour collection period.

Figure 1. Mean arterial pressure and the effects of vasopressin antagonism (dPMeTyrAVP) and converting enzyme inhibition (captopril) in adrenalectomized and control rats. Data are represented as means ± SEM. n indicates the size of each group. NS = not significant.
combined inhibition of the vascular action of vasopressin and of the renin-angiotensin system produced a sustained reduction in mean arterial pressure of 24 ± 3 mm Hg, p < 0.005, or 27% in adrenalectomized rats. In contrast, mean arterial pressure of the control group was unaltered by either antagonist.

Activation of the renin-angiotensin system in adrenal insufficiency may be due to hypovolemia secondary to mineralocorticoid deficiency. As the presence of hypovolemia in salt-fed adrenalectomized rats is controversial (see above), an increase in plasma renin activity may be attributed to reduced arterial pressure or to the lack of a possible direct inhibitory effect of vasopressin on renin release. Saralasin, an angiotensin II receptor antagonist, reduces arterial pressure in adrenalectomized dogs and in uninephrectomized adrenalectomized rats with or without Goldblatt hypertension. The present studies document a significant depressor effect of captopril, a converting enzyme inhibitor, in adrenalectomized rats with intact kidneys. The response to inhibition of the renin-angiotensin system with these two agents indicates the importance of elevated levels of plasma renin activity in maintaining blood pressure in adrenal insufficiency. Captopril was given after arterial pressure reduction by vasopressin blockade in the present experiments. The depressor action of dPMeTyrAVP may have led to renin release, thus enhancing the magnitude of the effect of captopril. However, the degree of arterial pressure reduction by captopril in our studies is well within the range observed by administration of saralasin to glucocorticoid or mineralocorticoid-deficient dogs.

In conclusion, the direct participation of vasopressin for maintenance of arterial pressure in experimental adrenal insufficiency in the rat has been demonstrated by use of a specific antagonist of the vascular action of this hormone. In addition, the activity of the renin-angiotensin system has been confirmed in this model. Thus, in adrenal insufficiency, a model of chronic hypotension, the two pressor peptide systems perform an important role in preventing further reduction of systemic arterial pressure.

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