Natriuretic Effect of Angiotensin in Dogs Revealed after Administration of Reserpine and Guanethidine

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ABSTRACT

In untreated dogs anesthetized with morphine-chloralose, a small dose of angiotensin (0.0375 μg/kg per min) produced a large reduction in renal blood flow, a smaller reduction in glomerular filtration rate and an antinatriuresis. A graded change in the excretion of sodium in response to angiotensin occurred after the successive administration of reserpine and guanethidine. In reserpine-treated dogs, angiotensin did not produce an antinatriuresis, whereas in the same dogs after giving guanethidine, a natriuresis occurred in response to angiotensin (from a control of 60 to 189 μEq Na/min). The effects of angiotensin on blood pressure and glomerular filtration rate were similar in all dogs. The renal vasoconstrictor effect of angiotensin was decreased in 3 of 8 dogs after guanethidine. At the high dose of angiotensin (0.375 μg/kg per min), the natriuresis was larger (from a control of 75 to 353 μEq Na/min) and was unrelated to changes in glomerular filtration rate or renal blood flow. In reserpine- and guanethidine-treated dogs, angiotensin increased sodium excretion sufficiently during renal arterial constriction to produce a large reduction in renal blood flow and the filtered load of sodium. These results suggest that angiotensin has a renal tubular action that is uncovered by sympathetic blockade produced by reserpine and guanethidine.

ADDITIONAL KEY WORDS

renal blood flow renal neurohumors filtered load of sodium

glomerular filtration rate angiotensin and salt excretion

sympathetic nervous system chloralose-anesthetized dogs

In normal man (1, 2) and in the dog (3), the intravenous administration of angiotensin II (henceforth referred to as angioten-
laboratory to be partially dependent upon an interaction with the sympathetic nerves. Thus, the renal vasoconstrictor action of angiotensin was blocked by renal denervation or by adrenergic blocking agents, such as guanethidine (9, 10). These studies suggested that blockade of the renal vascular activity of angiotensin by guanethidine might reveal a renal tubular action of angiotensin. However, the acute administration of guanethidine resulted in a sympathomimetic response which obscured any renal tubular effects of angiotensin that might have occurred. To circumvent the initial sympathomimetic effect of guanethidine, reserpine was administered 1 day prior to the study. In this way, it was possible to demonstrate a graded change in sodium excretion in response to angiotensin after the successive administration to the same dog of reserpine and guanethidine. Thus, reserpine prevented the antinatriuresis and guanethidine permitted the demonstration of a natriuresis on giving angiotensin. Inasmuch as the natriuresis produced by angiotensin after sympathetic blockade was not primarily determined by elevated blood pressure, increased filtered load of sodium or decreased renal vasoconstrictor action of angiotensin, a renal tubular action of angiotensin presumably determined the natriuresis.

Methods

Male mongrel dogs weighing 18 to 29 kg were anesthetized with morphine sulfate (2 mg/kg, subcutaneously) and chloralose (70 mg/kg, iv). Through a transverse abdominal incision, a renal artery (usually the left) was dissected free, care being taken not to disturb its innervation. A catheter was introduced in a retrograde direction into the ureter and secured in place at the ureteropelvic junction. A Sanborn multichannel direct writer was used to record mean aortic blood pressure as measured by a Statham transducer (Statham M-4001). An electromagnetic flowmeter transducer with an opening of 2.5 or 3.0 mm was placed on a renal artery. The electromagnetic flowmeter was calibrated by perfusing 0.9% saline through an excised renal arterial segment. In 3 in vivo experiments, inflow measured with the electromagnetic flowmeter was compared simultaneously over a wide range of renal blood flows with renal venous outflow measured by a Shipley-Wilson rotameter or directly by a T-tube inserted in the renal vein; the values agreed within 10%.

Saline (0.9%) was administered intravenously throughout the procedure at the rate of 6 to 8 ml/min; sufficient 1.5% creatinine (for measurement of GFR) was given to maintain a plasma level of 0.15 to 0.20 mg/ml. Mannitol was added
to the saline (final solution 1.5% mannitol) in all dogs 1 hr prior to the first experimental period, because, in most instances, the urine flows in reserpine-treated animals were low, i.e., less than 0.5 ml/min per kidney (11). Inasmuch as mean aortic blood pressure and renal blood flow were continuously recorded, any departure from the steady state was immediately apparent. The clearance period was unacceptable if changes in renal blood flow of ± 5% of the control renal blood flow occurred. Venous blood samples were removed at the midpoint of each collection period.

This experimental preparation eliminated the necessity for longer or more frequent clearance periods which may have resulted in the development of tachyphylaxis during an angiotensin infusion (5). All periods were of 10 to 12 min duration, except when the larger dose of angiotensin (0.375 μg/kg per min) was given, necessitating periods of 7 to 8 min duration. The gradual development of tachyphylaxis to the vasoconstrictor effect of the larger dose of angiotensin over an 11-min period is illustrated in Figure 1. If tachyphylaxis to the vasoconstrictor effect of angiotensin occurred, natriuresis would have resulted, thereby obscuring the effects of drug intervention. Tachyphylaxis to angiotensin was prevented by using doses of angiotensin (0.0375 μg/kg per min) which have been observed in dogs (5) (given the time limits of these experiments) not to produce tachyphylaxis (<0.050 μg/kg per min). If larger doses of angiotensin (0.375 μg/kg per min) were administered, the periods of infusion were shorter. In addition, intervals of at least 30 min were allowed between administration of angiotensin to the reserpine-treated dogs and to the same dogs after giving guanethidine. A period of this duration previously prevented the occurrence of tachyphylaxis (9). Angiotensin II (as the amide, Hypertensin Ciba) was infused intravenously in saline by a Braun infusion pump in appropriate concentration so as to deliver 0.375 ml/min.

Thirteen dogs received reserpine, 1 mg/kg im, and 0.5 mg/kg iv, 24 hr and 4 hr, respec-

![Figure 2](http://circres.ahajournals.org/)

The effect of intravenously administered angiotensin (0.0375 μg/kg per min) on mean blood pressure (MBP) and renal blood flow (RBF) in untreated dogs and in reserpine-treated dogs before and after guanethidine administration. On the left-hand side of each line plot are the control values (C), on the right-hand side are the values produced by intravenous infusions of angiotensin (A). For the untreated dogs, only the mean values and the standard errors of the mean are represented (dot-dash). For the reserpine-treated dogs, each experiment is indicated (solid line). The plots of the data for the reserpine-treated dogs after guanethidine administration are represented by interrupted lines. The mean values for the reserpine-treated dogs are indicated by the heavy solid line before guanethidine administration and the heavy interrupted line after guanethidine administration. The values for RBF are corrected to 1.0 m², body surface area.
ANGIOTENSIN AND NATRIURESIS

Effectively, before the experiment. Two dose levels of angiotensin were used—0.0375 and 0.375 μg/kg per min. Guanethidine was always given in a dose of 10 mg/kg iv. Five dogs, of which 2 were untreated, were given angiotensin before and after renal arterial constriction. For all dogs, there were two control periods and one period during which angiotensin was infused. After starting an angiotensin infusion, an interval of 3 to 5 min preceded beginning a collection period to allow stabilization of blood pressure and renal blood flow.

Sodium and potassium concentrations were determined by flame photometry (Coleman). Creatinine was analyzed by the method of Bonsnes and Taussky (12). Statistical analyses were made on paired measurements of control and experimental values and on the differences between the means of the same measurements using the Student t test (13). When each dog served as its own control, the Student t test for paired data was used. With separate controls, the Student t test for unpaired data was used. A P value of 0.05 or less was considered statistically significant. The filtered load of sodium was determined from the product of glomerular filtration rate and plasma sodium. Donnan corrections were not made for plasma sodium. Renal plasma flow for determination of filtration fractions (glomerular filtration rate/renal plasma flow) was calculated by: (1 minus hematocrit) x renal blood flow.

Results

EFFECTS OF ANGIOTENSIN (0.0375 μg/kg per min)

Figures 2 and 3 show the results of giving angiotensin intravenously to 5 untreated dogs and 8 reserpine-treated dogs before and after receiving guanethidine. Measurements during the control periods and the periods during which angiotensin was infused in Figures 2 through 4 are denoted by C and A, respectively. After observing the effects of angiotensin in the 8 reserpine-treated dogs, 10 mg/kg of guanethidine was given iv, control values obtained, and the angiotensin infusion was repeated (interrupted lines, right panels of Figs. 2 and 3). All values except mean aortic blood pressure are corrected to 1.0 m² of body surface area. In Figures 3 and 4, glomerular filtration rate (GFR), renal blood flow (RBF) and sodium excretion (U₁/V) represent the values determined simultaneously for 1 kidney.

Untreated dogs. The effects are shown in Table 1 and in Figures 2 and 3. Note that

![Figure 3](http://circres.ahajournals.org/)

The effect of intravenously administered angiotensin (0.0375 μg/kg per min) on glomerular filtration rate (GFR) and sodium excretion (U₁/V) in untreated dogs and reserpine-treated dogs before and after guanethidine administration. Legend as in Figure 2. The values for GFR and U₁/V are corrected to 1.0 m², body surface area.
urinary excretion of sodium decreased. Urine volume decreased from 3.2 to 2.3 ml/min \((P < 0.05)\) and potassium excretion changed variably. Urinary sodium concentration decreased while potassium concentration did not change; plasma sodium and hematocrit remained at the control values of 153 mEq/liter and 41, respectively.

**Reserpine-treated dogs.** The dogs receiving reserpine showed no change in sodium excretion (Fig. 3 and Table 1). There were no differences between the untreated and treated dogs in the changes produced by angiotensin in mean aortic blood pressure, renal blood flow, glomerular filtration rate, potassium excretion and urinary volume. The changes in sodium and potassium excretion and urinary volume were variable during an angiotensin infusion. The only reserpine-treated dog showing an increased renal blood flow to angiotensin (from a control of 112 to 152 ml/min) before giving guanethidine demonstrated an associated natriuresis (from a control of 17 to 74 \(\mu\)Eq/min). Sodium and potassium concentrations, and the percent sodium excreted of the filtered load of sodium were not significantly changed from their control values during an angiotensin infusion (Table 1).

**GUANETHIDINE ADMINISTRATION TO RESERPINE-TREATED DOGS**

After giving guanethidine to the reserpine-treated dogs, angiotensin infusion had a striking effect on sodium excretion, producing an invariable natriuresis from a control of 60 to 189 \(\mu\)Eq/min (Table 1 and Fig. 3). The natriuresis was accompanied by a kaliuresis and diuresis which distinguished the response of dogs receiving guanethidine from the untreated group and the same reserpine-treated dogs before guanethidine \((P < 0.01\) for differences between the guanethidine-treated dogs and the same dogs before guanethidine, as well as the untreated dogs in the changes in sodium and potassium excretion and urinary volume produced by angiotensin). The changes in renal blood flow further distinguished the response of the reserpine-treated dogs receiving guanethidine from reserpine-treated dogs before guanethidine and untreated dogs \((P < 0.05)\). Thus, mean reductions in renal blood flow of 42 and 33\% of control renal flow were produced by angiotensin in normal and reserpine-treated dogs, respectively, whereas after guanethidine, angiotensin produced a 20\% reduction in renal blood flow in reserpine-treated dogs (Table 1 and Fig. 2).
Table 1

Summary of Changes Produced by Angiotensin (0.0375 μg/kg per min) in 5 Normal Dogs and in 8 Reserpine-Treated Dogs before and after Guanethidine

<table>
<thead>
<tr>
<th></th>
<th>MA BP* (mm Hg)</th>
<th>RBF/m²* (ml/min)</th>
<th>GFR/m²* (ml/min)</th>
<th>U\textsubscript{Na}V/m²* (mEq Na/min)</th>
<th>U\textsubscript{K}V/m¹* (mEq K/min)</th>
<th>Urinary sodium concentration* (mEq/liter)</th>
<th>Urinary potassium concentration* (mEq/liter)</th>
<th>% Filtered sodium excreted†</th>
<th>Filtration fraction*</th>
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</thead>
<tbody>
<tr>
<td>Untreated dogs (5)</td>
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<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td>146 ± 13</td>
<td>221 ± 25</td>
<td>44 ± 4</td>
<td>113 ± 26</td>
<td>67 ± 17</td>
<td>64 ± 19</td>
<td>33 ± 5</td>
<td>2.12 ± 0.61</td>
<td>.30 ± .08</td>
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<tr>
<td>Angiotensin</td>
<td>179 ± 15</td>
<td>129 ± 11</td>
<td>40 ± 5</td>
<td>48 ± 6</td>
<td>53 ± 12</td>
<td>42 ± 12</td>
<td>30 ± 10</td>
<td>1.16 ± 0.26</td>
<td>.45 ± .07</td>
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<td>P value</td>
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<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
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<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
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<td>Reserpine-treated dogs (8) before guanethidine</td>
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</tr>
<tr>
<td>Control</td>
<td>98 ± 5</td>
<td>165 ± 18</td>
<td>27 ± 3</td>
<td>71 ± 18</td>
<td>33 ± 6</td>
<td>51 ± 10</td>
<td>26 ± 4</td>
<td>1.54 ± 0.31</td>
<td>.25 ± .03</td>
</tr>
<tr>
<td>Angiotensin</td>
<td>145 ± 4</td>
<td>110 ± 12</td>
<td>24 ± 3</td>
<td>54 ± 8</td>
<td>31 ± 5</td>
<td>43 ± 5</td>
<td>29 ± 5</td>
<td>1.55 ± 0.35</td>
<td>.35 ± .06</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
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<td></td>
<td>&lt; 0.05</td>
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<td>Reserpine-treated dogs (8) after guanethidine</td>
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<tr>
<td>Control</td>
<td>89 ± 6</td>
<td>130 ± 13</td>
<td>24 ± 3</td>
<td>60 ± 14</td>
<td>23 ± 4</td>
<td>56 ± 16</td>
<td>23 ± 3</td>
<td>2.07 ± 0.71</td>
<td>.30 ± .03</td>
</tr>
<tr>
<td>Angiotensin</td>
<td>137 ± 9</td>
<td>109 ± 16</td>
<td>24 ± 2</td>
<td>189 ± 37</td>
<td>39 ± 8</td>
<td>99 ± 24</td>
<td>21 ± 4</td>
<td>5.53 ± 1.20</td>
<td>.44 ± .06</td>
</tr>
<tr>
<td>P value</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
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<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.05</td>
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</tbody>
</table>

*The mean control values for all dogs were compared with the mean values resulting from angiotensin administration. Absence of P value indicates no statistical significance. All values except MA BP are the values determined for 1 kidney. MA BP = mean aortic blood pressure; RBF = renal blood flow in ml/min; GFR = glomerular filtration rate in ml/min; U\textsubscript{Na}V = urinary excretion of sodium in mEq/min; and U\textsubscript{K}V = potassium excretion in mEq K/min. RBF, GFR and U\textsubscript{Na}V were corrected to 1.0 square meter of body surface area (m²). Each value is given ±SEM.

†% Sodium excreted = \([U_{Na}V/\text{filtered load of Na (FNa})] \times 100.\)
blood pressure and changes in glomerular filtration rate induced by angiotensin were similar for all dogs.

The increase in sodium excretion occurred in the face of variable changes in glomerular filtration rate (Fig. 3). In 5 experiments the rate was unchanged, or changes of 2 ml/min or less were observed; in 2 experiments increases of 5 and 7 ml/min occurred and in 1 experiment there was a reduction of 12 ml/min. The changes in renal blood flow during an angiotensin infusion were variable and unrelated to the magnitude of the natriuresis; the flow increased in 3 experiments and decreased in 5 experiments (Fig. 2).

The increase in sodium excretion produced by angiotensin in these dogs was accounted for by both an increase in urinary volume and urinary sodium concentration (Table 1). Angiotensin resulted in each instance in an increased excretion of the filtered load of sodium (Table 1). Potassium concentration was not changed. The mean resting values in the reserpine-treated animals, before and after giving guanethidine, were 149 and 151 mEq/liter for plasma sodium, .40 and .38 for hematocrit and .25 and .30 for filtration fraction, respectively, none of which represents a significant change.

EFFECTS OF ANGIOTENSIN (0.375 µg/kg per min)

Five untreated dogs and 5 dogs that received reserpine and guanethidine were given angiotensin in a dose tenfold that in the experiments just described. In Figure 4, the results of the 10 experiments and their means are shown. The effects of the large dose of angiotensin in the reserpine-treated dogs were not determined before guanethidine administration, to prevent the development of tachyphylaxis to angiotensin.

Untreated dogs. The larger dose of angiotensin produced an elevation in arterial blood pressure from 133 to 202 mm Hg (P < 0.01), a reduction in renal blood flow from 185 to 88 ml/min (P < 0.001) and in glomerular filtration rate from 40 to 23 ml/min (P < 0.05), and simultaneously retention of sodium and potassium and an antidiuresis (P < 0.05 for changes in sodium and potassium excretion and urinary volume) (Fig. 4). The most striking differences between the small and large doses of angiotensin in untreated dogs were the greater reductions in glomerular filtration rate and elevations in arterial blood pressure produced by the larger doses (Figs. 2, 3 and 4).

Reserpine- and guanethidine-treated dogs. The most noteworthy finding was, as in the reserpine- and guanethidine-treated dogs given the smaller dose of angiotensin, a marked natriuresis in response to angiotensin infusions. From a control sodium excretion of 75 µEq/min, an increase to 353 µEq/min occurred during angiotensin infusion (P < 0.01). The coincident changes in glomerular filtration rate were variable. Thus, in the face of reductions of glomerular filtration rate of 11 and 5 ml/min, a natriuresis was observed of a magnitude similar to that which occurred with an unchanged or increased glomerular filtration rate. Simultaneously, angiotensin raised arterial blood pressure from a control of 105 to 198 mm Hg (P < 0.001) and produced a kaluresis and diuresis (Fig. 4). A significant difference between the untreated and treated dogs was noted for changes produced by angiotensin in sodium and potassium excretion and urinary volume (P < 0.01 for each). Angiotensin produced changes in glomerular filtration rate, renal blood flow, and arterial pressure which did not differ significantly between the two groups.

The increases in sodium excretion produced by angiotensin represented excretions of 6.0, 8.8, 8.9, 10.7 and 14.6% of the filtered load of sodium, while urinary sodium concentration increased by 44, 68, 36, 42 and 32 mEq/liter, respectively.

EFFECTS ON CONSTRICTION OF THE RENAL ARTERY ON THE RESPONSE TO ANGIOTENSIN

To dissociate further the changes in glomerular filtration rate and renal blood flow from the simultaneous natriuresis produced by angiotensin, the rate and flow were decreased by renal arterial constriction in 3 reserpine-treated dogs (Table 2). Guanethidine was given 10 min after completion of the first angiotensin infusion when the renal blood
### TABLE 2

Effects of Angiotensin on Renal Blood Flow, Glomerular Filtration Rate, Sodium and Potassium Excretion during Constriction of the Left Renal Artery of the Reserpine-Treated Dog

<table>
<thead>
<tr>
<th>Duration (min)</th>
<th>Aortic blood pressure (mm Hg)</th>
<th>RBF (ml/min)</th>
<th>GFR (ml/min)</th>
<th>V (ml/min)</th>
<th>U_NaV (µEq/min)</th>
<th>U_KV (µEq/min)</th>
<th>P_Na (µEq/min)</th>
<th>% of filtered Na excreted</th>
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<tr>
<td><strong>Dog 1, 19 kg</strong></td>
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<tr>
<td>Control 1</td>
<td>10</td>
<td>90</td>
<td>224</td>
<td>39</td>
<td>0.52</td>
<td>9</td>
<td>27</td>
<td>158</td>
</tr>
<tr>
<td>Control 2</td>
<td>10</td>
<td>90</td>
<td>213</td>
<td>36</td>
<td>0.60</td>
<td>10</td>
<td>29</td>
<td>156</td>
</tr>
<tr>
<td>Angiotensin (0.0375 µg/kg per min)</td>
<td>10</td>
<td>125</td>
<td>128</td>
<td>32</td>
<td>0.63</td>
<td>9</td>
<td>23</td>
<td>154</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>65</td>
<td>164</td>
<td>35</td>
<td>1.03</td>
<td>20</td>
<td>21</td>
<td>153</td>
</tr>
<tr>
<td>Renal arterial constriction</td>
<td>11</td>
<td>110</td>
<td>66</td>
<td></td>
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<tr>
<td>Angiotensin (0.0375 µg/kg per min)</td>
<td>10</td>
<td>175</td>
<td>74</td>
<td>26</td>
<td>1.17</td>
<td>19</td>
<td>20</td>
<td>150</td>
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<td><strong>Dog 2, 18.5 kg</strong></td>
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<tr>
<td>Control 1</td>
<td>10</td>
<td>100</td>
<td>160</td>
<td>19</td>
<td>0.54</td>
<td>13</td>
<td>17</td>
<td>160</td>
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<tr>
<td>Control 2</td>
<td>10</td>
<td>100</td>
<td>151</td>
<td>19</td>
<td>0.51</td>
<td>10</td>
<td>17</td>
<td>160</td>
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<tr>
<td>Angiotensin (0.0375 µg/kg per min)</td>
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<td>135</td>
<td>89</td>
<td>18</td>
<td>0.43</td>
<td>13</td>
<td>15</td>
<td>157</td>
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<tr>
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<td>11</td>
<td>105</td>
<td>105</td>
<td>25</td>
<td>0.87</td>
<td>68</td>
<td>29</td>
<td>158</td>
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<tr>
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<td>65</td>
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<tr>
<td>Angiotensin (0.0375 µg/kg per min)</td>
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<td>165</td>
<td>60</td>
<td>21</td>
<td>1.10</td>
<td>179</td>
<td>26</td>
<td>160</td>
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<td><strong>Dog 3, 24 kg</strong></td>
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<tr>
<td>Control 1</td>
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<td>85</td>
<td>248</td>
<td>45</td>
<td>0.61</td>
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<tr>
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<td>42</td>
<td>0.66</td>
<td>20</td>
<td>27</td>
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<tr>
<td>Angiotensin (0.0375 µg/kg per min)</td>
<td>10</td>
<td>160</td>
<td>167</td>
<td>39</td>
<td>0.60</td>
<td>19</td>
<td>23</td>
<td>162</td>
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<tr>
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<td>100</td>
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<td>36</td>
<td>0.58</td>
<td>43</td>
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<tr>
<td>Renal arterial constriction</td>
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<td>103</td>
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<td></td>
</tr>
<tr>
<td>Angiotensin (0.0375 µg/kg per min)</td>
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<td>220</td>
<td>65</td>
<td>28</td>
<td>1.63</td>
<td>125</td>
<td>21</td>
<td>157</td>
</tr>
</tbody>
</table>

RBF = renal blood flow, GFR = glomerular filtration rate, V = urine flow, U_NaV = excreted sodium, U_KV = excreted potassium, and P_Na = plasma sodium. The amount of guanethidine given was 10 mg/kg iv.
flow and aortic blood pressure had returned to control levels. In dog 2 of Table 2, angiotensin did not produce a natriuresis before giving guanethidine. Constriction of the left renal artery reduced blood flow from 105 to 65 ml/min. During renal arterial constriction, an angiotensin infusion resulted in an increase of sodium excretion from 68 to 179 μEq/min (representing the excretion of 5.33% of the filtered load), although the filtration rate and blood flow decreased below the control values.

In dog 3, constriction of the renal artery resulted in a reduction of renal blood flow from 186 to 103 ml/min. Infusion of angiotensin during renal arterial clamping produced an increase of sodium excretion from 43 to 125 μEq/min, in spite of a reduction of glomerular filtration rate from a control of 36 to 28 ml/min and a further reduction of renal blood flow from 103 to 65 ml/min.

In dog 1, renal blood flow and glomerular filtration rate were decreased more during renal arterial constriction (60% and 26%, respectively) than in dog 2 (38% and 16%) and dog 3 (45% and 22%); this probably accounts for the failure to demonstrate a natriuresis in response to an angiotensin infusion. However, the sodium excretion during the angiotensin infusion after arterial constriction was twice that during the angiotensin infusion before guanethidine administration, in spite of a reduction in filtration rate from 35 to 26 ml/min.

In 2 experiments, giving angiotensin (0.0375 μg/kg per min) to untreated dogs during renal arterial constriction did not result in a natriuresis. Renal blood flow decreased 49 and 60% and filtration rate decreased 32 and 76% during renal arterial constriction, but sodium excretion fell from 54 to 9 μEq/min in 1 and was unchanged in the other.

Discussion
A graded effect on changes in salt excretion produced by angiotensin occurred in response to the successive administration of reserpine and guanethidine. Thus, in the untreated dogs, angiotensin produced an antinatriuresis; in reserpine-treated dogs, angiotensin produced no change in sodium excretion, but in the same reserpine-treated dogs also given guanethidine, angiotensin produced a consistent natriuresis.

The possible determinants of the natriuresis produced by angiotensin after giving guanethidine were hemodynamic or tubular; either may be associated with the participation of a neurohumor or hormone in influencing sodium excretion. Natriuresis was described in dogs to result from elevations of renal arterial pressure (14, 15). A natriuresis produced by angiotensin, when its renal vasoconstrictor action was blunted by vasodilator agents, was mainly attributed to its simultaneous pressor effect by Earley and Friedler (6). The present work did not allow such interpretation because similar elevations in blood pressure occurred in response to angiotensin in all groups of dogs, in spite of dissimilar effects on sodium excretion (Figs. 2, 3 and 4).

Attenuation of its vasoconstrictor effect was not essential to the natriuresis produced by angiotensin. Thus, a mean reduction of renal blood flow of 36% in response to the larger dose of angiotensin occurred simultaneously with a fivefold increase of sodium excretion (Fig. 4). Additionally, during constriction of the renal artery, reductions of blood flow of 43 and 65% in response to angiotensin did not prevent a concomitant natriuresis (dogs 2 and 3, Table 2). Changes in blood flow or perfusion pressure may have facilitated the angiotensin-induced natriuresis, but they were not essential to its demonstration. Furthermore, the observation of Earley and Friedler (6) suggested that altered distribution of blood flow within the kidney is not a determinant of the natriuresis produced by angiotensin.

Changes in glomerular filtration rate were not necessary for the natriuresis produced by angiotensin, which corresponds to the findings in subjects having hypertension or cirrhosis with ascites (2, 16). A clear separation of filtration rate from sodium excretory

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changes was provided by the effects of the higher dose of angiotensin (Fig. 4) and by giving angiotensin during renal arterial constriction (Table 2). Thus, in 5 experiments in reserpine- and guanethidine-treated dogs, 0.375 μg/kg per min of angiotensin produced similar large increases in sodium excretion, while filtration rate either increased (by 6 and 9 ml/min), was unchanged, or decreased (by 5 and 11 ml/min). Reductions in glomerular filtration rate of 16 and 22% in response to angiotensin during renal arterial constriction were associated with large increases in sodium excretion in response to angiotensin (Table 2).

Since the natriuresis induced by angiotensin after guanethidine administration cannot be related to changes in glomerular filtration rate, renal blood flow, or blood pressure, a renal tubular effect of angiotensin emerges as a sufficient explanation for the natriuresis. A physiologic action of angiotensin on the transport of sodium by the renal tubules was advanced by Leyssac (8, 17). The proposal of Rector et al. (18), that altered renal tubular geometry consequent to changes in intratubular pressure determines proximal tubular sodium absorption as an alternative explanation for the angiotensin-induced natriuresis, cannot be excluded by the present investigation. Thus, modification by sympathetic blockade of the changes in renal segmental vascular resistances in response to angiotensin could alter the intratubular pressure, thereby influencing sodium excretion.

The conversion of the angiotensin-induced antinatriuresis to natriuresis by sympathetic blockade suggested an interaction of angiotensin with the sympathetic nervous system. An interrelationship of sympathetic nervous activity and angiotensin was shown for several neuro-effector sites (9, 19). Sympathetic blockade may have modified at least one element of the effect of administered angiotensin, namely, release of catecholamines or other neurohumors which might have obscured a direct action of angiotensin on the renal tubules. An antinatriuretic effect of adrenergic nervous activity or injected catecholamines was described (20-22). Angiotensin was reported to release catecholamines from the adrenal medulla (23). Guanethidine was demonstrated to prevent the release of catecholamines from tissue stores by agents having an effect either on the adrenergic storage vesicle or the neuronal membrane (24, 25). However, guanethidine administered intravenously will itself release catecholamines, unless prevented by reserpine-treatment (26). Furthermore, guanethidine was reported to promote sodium excretion by adrenergic blockade under several conditions: in normal man subject to sodium deprivation (27); and in normal subjects given saline infusions or treated with sodium-retaining steroids (20). Reserpine alone was not sufficient to uncover the natriuretic action of angiotensin in the present experiments since the catecholamines of the kidneys are less susceptible to depletion by reserpine than those of the spleen or heart (28); and the amount of reserpine required to produce this effect would seriously compromise the renal circulation and excretion of salt (9, 11). Reserpine was reported to produce, in rats, an antidiuresis related in large part to ADH release (11). The reduced glomerular filtration rate seen after reserpine treatment in the present experiments provides an additional explanation for the antidiuresis (Table 1).

Of primary importance to any effect of angiotensin on the kidney is the initial site of its action. Under physiologic conditions, angiotensin was suggested to have a predominantly postglomerular effect (29), which could promote sodium excretion by a direct effect on the renal tubules (2, 8, 16, 17), or increase glomerular filtration rate by constriction of the efferent glomerular arteriole, or both. In contrast, angiotensin, irrespective of the route of administration (intravenous or renal intraarterial), must have an initial preglomerular site of action. Administration of angiotensin, in contradistinction to endogenous angiotensin, may release catecholamines and perhaps other neurohumors from all of the preglomerular arterial elements (as
well as the adrenal medulla in the case of intravenous administration), thus accounting for the antinatriuresis of injected angiotensin.

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References


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