MANDEL, Morris Jack, 1929—
EFFECT OF ANGIOTENSIN INFUSION ON REGIONAL BLOOD FLOW AND REGIONAL VASCULAR RESISTANCE IN THE RAT.

The Ohio State University, Ph.D., 1962
Physiology

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flow is known to be significantly reduced in the chronic renal hypertensive animal and in the hypertensive individual, the effects in the early hypertensive state are relatively unknown. However, White et al have shown that rats made hypertensive by a choline or potassium deficient diet have normal renal hemodynamics (13). Coronary flow is increased in the renal hypertensive dog as is cardiac muscle mass, the flow per unit weight of heart tissue being similar to that found in the normal animal (18). Cerebral flow is stated to be unchanged (19).

The cardiac output presents a different problem. According to Freis (20) there is a high cardiac output-normal peripheral resistance type hypertension, and a normal cardiac output-high peripheral resistance type hypertension in man. Whether either one is of renal origin is not known, but the fact that both states are associated with a clinically indistinguishable hypertensive condition raises the question of which one, if either, may be due to the renin-angiotensin system. The fact that two-thirds of the dogs that develop spontaneous hypertension have renal lesions at necropsy points up the question even further (21).

Following the isolation and subsequent synthesis of angiotensin II, (16, 22-24) biologically and chemically similar to animal angiotensin, the hemodynamic effects of this agent could be more easily evaluated. The agent most frequently implicated in the possible renal mechanism of hypertension is angiotensin. Since most studies purporting to demonstrate that angiotensin may be the causative agent are based on the similarity of the hemodynamic effects of angiotensin and hypertension, the comparisons of which are few, it is felt desirable and
useful to make a systematic exploration of the hemodynamic effects of angiotensin in preparation for a further study in which the effects of angiotensin infusion and renal hypertension of varying durations can be compared.

The physiology, and pharmacology of angiotensin have been comprehensively reviewed by Page and Bumpus (25). As these authors point out, the results of older investigations may have been misleading due to the use of impure preparations or invalid techniques.

Although the literature is replete with information on its overall pressor properties and its effects on the cardiac output, the effects of angiotensin on regional blood flow and organ vascular resistance have not been adequately evaluated for most regions.

There are some observations on the effects of angiotensin on blood flow in isolated perfused organs and more recently on organs in situ. Such observations have been confined to a few organs or areas. The changes which are seen are difficult to evaluate properly in perspective due to the fact that changes in the rest of the circulation occurring simultaneously are not measured.

A method developed in the Department of Physiology at The Ohio State University allows the measurement of the distribution of the cardiac output to all organs simultaneously (26). This has made it possible to assess more completely the effects of angiotensin on regional blood flow and regional vascular resistance. The present
report\(^1\) is concerned with the application of this method to the
determination of regional blood flow and regional vascular resistance
in rats anesthetized with sodium pentobarbital and infused with
varying doses of angiotensin.

\(^1\)The material on which this dissertation is based, has been
accepted and published in the May, 1962, issue of Circulation Research.
Reproduction of this material in this dissertation has been authorized
by the editors of the above journal.
METHODS

Two hundred and eleven female rats of the Sprague-Dawley strain which had been fasted for 18 hours were used in these studies. Of these, 75 (51 control, 24 experimental) were used in blood pressure measurements; 38 (10 control, 28 experimental) were employed in cardiac output measurements; regional flow distribution for all organs other than the brain was measured in 71 (22 control, 49 experimental), and cerebral flow fraction was measured in 102 (37 control, 65 experimental).

Water was allowed ad libitum. The animals weighed between 190 and 250 Gm. Sodium pentobarbital (40 mg./Kg., intraperitoneally) was used as the anesthetic.

The angiotensin used was supplied by the Ciba Company in the powdered form of Valyl-5-angiotensin II. The powder was diluted with saline to appropriate dilutions, and infused into the femoral vein by means of a Harvard infusion pump for 5 to 25 minutes at 0.05 ml./min. of solution containing concentrations of angiotensin sufficient to yield 0.05, 0.1, 0.2, 0.3, 0.4, or 0.5 ug./Kg. body weight/min.

Mean arterial pressures were measured from the femoral artery in heparinized rats with a mercury manometer connected to the artery by a 25- or 27- gauge needle. The same animals were also used for either the cardiac output or the regional flow determination. Cardiac outputs
were measured by the indicator-dilution technique, using Rb\(^{86}\) as the indicator. The rate of sample collection was 60/min. Because of the hemorrhage produced by the cardiac output determination, different animals were used for regional blood flow studies.

Distribution of blood flow was measured by the indicator-fractionation technique employing Rb\(^{86}\) for all organs except the brain, and iodo\(^{131}\) antipyrine for the brain. The principle of the method has been discussed elsewhere (26, 27). The sacrificing times used were 30 and 60 seconds. No significant change in label uptake was noted between these times in any organ except the brain of angiotensin-infused animals. The method of handling the brain data will be discussed later. Blood flow values were obtained from the indicator fractions, when these were unchanged with time, by multiplying that fraction by the cardiac output of similarly treated animals. The resistance values were calculated as the ratio between the mean arterial pressure in dynes/cm\(^2\) and the derived flow values in cm\(^3\)/sec. All flow values were adjusted to those for a 200 Gm. rat.
RESULTS

CARDIAC OUTPUT, BLOOD PRESSURE, AND TOTAL PERIPHERAL RESISTANCE

The cardiac output of control rats in this colony has averaged 231 ml/kg/min. with a standard deviation of 43 ml/kg/min. over the last two years. In the present experiment, 10 rats receiving saline infusions had a slightly higher value for the cardiac output (278/73). In experiments in which no infusion was employed, cardiac outputs did not vary from the infusion group. Since the range of values obtained was very great and the difference from the mean of untreated controls was not statistically significant (p > 0.05), the colony control value was used in the calculation of blood flows.

Rats infused with angiotensin had substantially the same cardiac output as the untreated controls. Six rats, infused with 0.05 ug/kg/min., showed a cardiac output of $231 \pm 45$ ml/kg/min.; at 0.2 ug/kg/min., the value was $263 \pm 94$ in eight rats; at 0.3 ug/kg/min., the value was $205 \pm 42$ in six rats; at 0.5 ug/kg/min., the cardiac outputs averaged $250 \pm 60$ ml/kg/min. in eight rats. Intermediate doses were not explored. The average of the experimental group was 240 ml/kg/min. When the infusion values are compared to the controls, the differences are not statistically significant (p > 0.05).
The blood pressure values at equilibrium, which always occurred within 3½ minutes, were as follows: at the infusion rate of 0.05 ug/kg/min. (6 animals), the value was unchanged from the control value of 113 ± 16 mm, Hg (51 animals). Blood pressures at higher infusion rates were: 0.1, 161 ± 6 mm, Hg (2 rats); 0.3, 160 ± 5 mm, Hg (2 rats); 0.4, 169 ± 8 mm, Hg (3 rats); 0.5, 168 ± 6 mm, Hg (9 rats). The average of the experimental group, excluding the lowest concentration, where no change in the blood pressure was noted, was 164 ± 5 mm, Hg. The difference in blood pressure was statistically significant (p < 0.05).
The fractional distribution of blood flow is tabulated in Table 1. It will be noted that the flow fraction to the heart, lungs and brain is significantly increased by angiotensin. A significant increase is also noted to the adrenal gland, but only at the lowest infusion level (0.05 ug/kg/min.). Although an apparent increase is seen at the other infusion levels, these changes are not significant due to the large standard deviations. The same applies to those values in the spleen. Despite the lack of significance, the elevation seen in the splenic values is consistent, unlike those seen in the liver, gut and skin. The flow fraction to the carcass is also consistently elevated, but to a much lesser degree, the change being significant only at the highest infusion level. The only organ which shows a consistent and significant fall in flow fraction is the kidney, which is reduced at least 40 per cent, except at the lowest infusion rate.

Because of the decrease in label content of the brain with time, the values presented are those obtained by extrapolation to 10 seconds; the rationale for this treatment of the data is presented elsewhere (27). Extrapolation was not necessary with the other organs since no changes were noted in the two sacrificing times used in the experiment.

Table 2 describes blood flow to the organs. The values are obtained by multiplying the values in Table 1 by the cardiac output.
TABLE 1

FLOW FRACTIONS OF THE CARDIAC OUTPUT TO ORGANS OF RATS RECEIVING INFUSIONS OF ANGIOTENSIN BY VEIN

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>2.5±0.5b(22)c</td>
<td>2.9±0.3(6)</td>
<td>d3.5±0.7(6)</td>
<td>d3.3±0.4(6)</td>
<td>d4.1±0.6(7)</td>
<td>d4.3±0.7(7)</td>
<td>d3.8±0.7(17)</td>
</tr>
<tr>
<td>Lung</td>
<td>2.6±0.8(22)</td>
<td>2.8±0.3(6)</td>
<td>d3.9±0.8(6)</td>
<td>d3.7±0.8(6)</td>
<td>d4.4±1.4(7)</td>
<td>d4.2±0.8(7)</td>
<td>d3.5±0.9(17)</td>
</tr>
<tr>
<td>Kidney</td>
<td>17.8±2.8(22)</td>
<td>17.3±2.1(6)</td>
<td>d11.5±3.5(6)</td>
<td>d10.1±1.9(6)</td>
<td>d11.0±2.6(7)</td>
<td>d9.1±1.9(7)</td>
<td>d8.8±2.5(17)</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.00±0.1h(22)</td>
<td>1.32±0.12(6)</td>
<td>1.40±0.15(6)</td>
<td>1.30±0.10(6)</td>
<td>1.30±0.23(7)</td>
<td>1.30±0.30(7)</td>
<td>1.15±0.58(17)</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0.20±0.10(22)</td>
<td>0.34±0.02(6)</td>
<td>0.28±0.10(6)</td>
<td>0.25±0.15(6)</td>
<td>0.29±0.15(7)</td>
<td>0.32±0.13(7)</td>
<td>0.19±0.10(17)</td>
</tr>
<tr>
<td>Liver</td>
<td>6.8±1.9(22)</td>
<td>7.6±1.3(6)</td>
<td>8.8±1.4(6)</td>
<td>6.8±1.5(6)</td>
<td>10.1±3.0(7)</td>
<td>9.2±1.2(7)</td>
<td>6.7±1.2(17)</td>
</tr>
<tr>
<td>Gut</td>
<td>18.6±5.3(22)</td>
<td>20.2±2.5(6)</td>
<td>20.9±2.4(6)</td>
<td>18.5±5.2(6)</td>
<td>19.1±2.2(7)</td>
<td>19.7±3.9(7)</td>
<td>17.6±4.8(17)</td>
</tr>
<tr>
<td>Skin</td>
<td>6.7±1.5(22)</td>
<td>8.5±1.7(6)</td>
<td>7.6±2.1(6)</td>
<td>8.1±1.9(6)</td>
<td>6.7±1.4(7)</td>
<td>7.2±1.7(7)</td>
<td>6.9±2.0(17)</td>
</tr>
<tr>
<td>Carcass</td>
<td>8.5±3.7(22)</td>
<td>10.1±4.7(6)</td>
<td>39.2±3.9(6)</td>
<td>39.3±3.7(6)</td>
<td>44.1±7.6(7)</td>
<td>43.5±7.3(7)</td>
<td>41.7±3.7(17)</td>
</tr>
<tr>
<td>Brain</td>
<td>1.37±0.37(37)</td>
<td>2.17±0.26(16)</td>
<td>d2.77±0.36(21)</td>
<td>&lt;--------</td>
<td>d2.21±0.28(12)</td>
<td>&lt;--------</td>
<td>d2.27±0.31(16)</td>
</tr>
</tbody>
</table>

*a All values as percentage of cardiac output

*b Standard deviations

*c Numbers in parenthesis refer to the number of animals used in each determination

*d p < 0.05
## TABLE 2  
**REGIONAL BLOOD FLOWS TO ORGANS OF RATS RECEIVING INFUSIONS OF ANGIOTENSIN BY VEIN**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>0.05(\text{ug/kg/min.})</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>1.15±0.10(^b)</td>
<td>1.34±0.13</td>
<td>d1.62±0.32</td>
<td>d1.52±0.17</td>
<td>d1.89±0.28</td>
<td>d1.99±0.30</td>
<td>d1.76±0.16</td>
</tr>
<tr>
<td>Lung</td>
<td>1.20±0.15</td>
<td>1.29±0.16</td>
<td>d1.80±0.39</td>
<td>d1.71±0.37</td>
<td>d2.03±0.66</td>
<td>d1.94±0.32</td>
<td>d1.62±0.23</td>
</tr>
<tr>
<td>Kidney</td>
<td>7.76±0.28</td>
<td>7.99±1.02</td>
<td>d5.31±1.70</td>
<td>d4.67±0.90</td>
<td>d5.08±1.13</td>
<td>d4.20±0.82</td>
<td>d4.07±0.59</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.46±0.08</td>
<td>0.61±0.08</td>
<td>0.65±0.12</td>
<td>0.60±0.12</td>
<td>0.60±0.10</td>
<td>0.60±0.13</td>
<td>0.53±0.14</td>
</tr>
<tr>
<td>Adrenal</td>
<td>0.092±0.020</td>
<td>0.157±0.030</td>
<td>0.129±0.040</td>
<td>0.116±0.070</td>
<td>0.134±0.060</td>
<td>0.148±0.060</td>
<td>0.088±0.030</td>
</tr>
<tr>
<td>Liver</td>
<td>3.11±0.40</td>
<td>3.51±0.63</td>
<td>4.07±0.66</td>
<td>3.11±0.71</td>
<td>4.67±0.128</td>
<td>4.25±0.51</td>
<td>3.10±0.32</td>
</tr>
<tr>
<td>Gut</td>
<td>8.59±1.07</td>
<td>9.33±1.30</td>
<td>9.66±1.16</td>
<td>8.55±2.51</td>
<td>8.82±0.95</td>
<td>9.10±1.53</td>
<td>8.13±1.28</td>
</tr>
<tr>
<td>Skin</td>
<td>3.10±0.33</td>
<td>3.93±0.84</td>
<td>3.51±1.04</td>
<td>3.71±0.90</td>
<td>3.10±1.06</td>
<td>3.33±0.75</td>
<td>3.19±0.55</td>
</tr>
<tr>
<td>Carcass</td>
<td>17.79±0.77</td>
<td>18.53±2.43</td>
<td>18.11±1.83</td>
<td>18.16±1.81</td>
<td>20.37±3.24</td>
<td>20.10±3.07</td>
<td>19.27±0.99</td>
</tr>
<tr>
<td>Brain</td>
<td>0.63±0.054</td>
<td>d1.00±0.121</td>
<td>d1.28±0.167</td>
<td>d1.02±0.125</td>
<td>------------</td>
<td>------------</td>
<td>d1.04±0.15</td>
</tr>
</tbody>
</table>

\(^a\)All values referred to 200 Gm. rat. Values as ml./min./organ: 231 ml./kg/min. taken as cardiac output for all animals.

\(^b\)Standard deviations

\(d_p < 0.05\)

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\(\text{Control}\) value indicated by \(d\) precedes time values showing statistically significant increase relative to control.
EFFECT OF ANGIOTENSIN INFUSION
ON REGIONAL BLOOD FLOW AND REGIONAL VASCULAR
RESISTANCE IN THE RAT

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Morris Jack Mandel, B. S., M. D.

The Ohio State University
1962

Approved by

Leon D. Epstein
Adviser
Department of Physiology
In the case of control animals, the value used was based on the colony average rather than those observed in the controls run with the experiment. All animals receiving angiotensin were assumed to have the same cardiac output. Since the average for the experimental group did not differ significantly from the colony average, the latter was used for both the control and experimental groups. Justification for this assumption will be presented later.

Since the cardiac output does not change, the flow values closely parallel those for the flow fraction. With angiotensin, blood flow is increased significantly to the heart and lungs at all levels above 0.05 ug/kg/min., to the brain at all levels and to the adrenal gland only at 0.05 ug/kg/min. Since the flow values are calculated from the flow fractions, the levels of confidence are as good as those seen in Table 1. Therefore, the increase seen in the spleen and in the adrenal at other infusion rates are only apparent. The fall in renal blood flow is marked, as would be expected from the flow fraction values.
REGIONAL RESISTANCE

Calculated resistances for various regions are shown in Table 3. Coronary and bronchial resistances are unchanged. The cerebral vascular resistance is reduced by low infusion levels, particularly at 0.05 and 0.1 ug/kg/min., and appears to approach control values at the higher levels. Because of the small number of animals used to explore the 0.2 - 0.4 ug/kg/min. infusion range, and the relative constancy of the results in this range, these values were grouped together.

It is noteworthy that, with the exception of a fall in adrenal and splenic resistance, this reduction in cerebral vascular resistance is observed at a dose level where there are no changes in any other measured parameter.

The resistances of the gut, skin, and carcass are increased to approximately the same extent by angiotensin at all doses above 0.05 ug/kg/min. Although the results are variable, in general, angiotensin produces about a 40 per cent increase in resistance in these areas. This is similar to the increase in total peripheral resistance. The adrenal resistance is significantly reduced by angiotensin at 0.05 ug/kg/min. with an apparent increase by 0.50 ug/kg/min. Intermediate doses are without any characteristic effect. Hepatic resistance fluctuates in a random manner. The largest resistance changes observed
TABLE 3

TERRITORIAL RESISTANCES OF RATS RECEIVING INFUSIONS OF ANGIOTENSIN BY VEIN

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control</th>
<th>Infusion Rate (ug/kg/min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Heart</td>
<td>79±11b</td>
<td>68±7</td>
</tr>
<tr>
<td>Lung</td>
<td>76±22</td>
<td>70±9</td>
</tr>
<tr>
<td>Kidney</td>
<td>11.7±2.4</td>
<td>11.3±1.3</td>
</tr>
<tr>
<td>Spleen</td>
<td>197±73</td>
<td>149±15</td>
</tr>
<tr>
<td>Adrenal</td>
<td>985±748</td>
<td>577±115</td>
</tr>
<tr>
<td>Liver</td>
<td>29±8</td>
<td>26±4</td>
</tr>
<tr>
<td>Gut</td>
<td>10.5±1.4</td>
<td>9.7±1.2</td>
</tr>
<tr>
<td>Skin</td>
<td>29±7</td>
<td>23±5</td>
</tr>
<tr>
<td>Carcass</td>
<td>5.1±0.5</td>
<td>4.9±0.2</td>
</tr>
<tr>
<td>Brain</td>
<td>14±4.5</td>
<td>91±18</td>
</tr>
<tr>
<td>TPR</td>
<td>1.96</td>
<td>1.96</td>
</tr>
</tbody>
</table>

All values as dynes sec./cm.² and adjusted for organs of 200 Gm. rat.

Standard deviation

Resistances of rats in 0.1 through 0.5 infusion series based on average blood pressure value for the group. (See text)

dp < 0.05
were those in the kidney where doses of 0.1 to 0.5 ug/kg/min. increased the resistance from more than 100 to more than 200 per cent. At 0.05 ug., the renal resistance was not affected.
DISCUSSION

Most of the pharmacological properties of angiotensin have been studied after single injections of the agent. In our experience, single injections give results which vary with the status of the blood pressure at the time of observation and are radically different from those obtained in constant infusion experiments. No attempt will be made to present these results here. It is important in comparing our experiments with those reported by previous investigators to recognize that our findings were based on constant infusions and that arterial blood pressures were at equilibrium when observations were made. It should be stressed further that the method employed here measures only functional blood flow, i.e., the blood which "services" the tissues; shunt flow will not be measured; however, the magnitude of shunt flow in the rat is probably inconsiderably small under the experimental conditions employed (26).
CARDIAC OUTPUT, BLOOD PRESSURE, AND TOTAL PERIPHERAL RESISTANCE

The cardiac output did not change in these experiments when angiotensin was administered by a constant infusion. However, the blood pressure and total peripheral resistance were increased about 45 per cent.

In heart-lung preparations, Hill and Andrus (28) noted a rise in the cardiac output in many experiments after an injection of angiotensin. In experiments on intact animals, most authors are in agreement that angiotensin does not influence the cardiac output. Maxwell et al (29) and Eckert and Rose (30) noted no change in cardiac output during angiotensin infusion in intact dogs using the Fick method. Potgieter et al (31, 32) noted similar results. Barer (33) used synthetic angiotensin and measured blood flow with the electromagnetic flowmeter. She found no consistent change in the cardiac output. In human studies, Schales et al (34), using the acetylene method, and Sancetta (35), using the Fick method, also found no changes in the output of the heart.

A few reports have appeared which suggest that angiotensin reduces the cardiac output. Page and Olmstead (quoted in Page and Bumpus (25), using the electromagnetic flowmeter, noted a fall in the cardiac output, stroke volume, and heart rate in intact unanesthetized dogs; Middleton and Wiggers (36), using cardiology, found a reduction
in cardiac output secondary to bradycardia after small doses of angiotensin and a reduction in cardiac output secondary to a fall in stroke volume after large doses. All angiotensin was given as a single injection and the data do not indicate that the fall in cardiac output was significantly large. A number of reports have appeared which suggest that the cardiac output may be reduced in man after angiotensin. Most of these are based on the ballistocardiogram. Taylor and Page (37) found angiotensin infusions in six normal subjects to reduce cardiac output 34 per cent. Wilkins and Duncan (38) found a 33 per cent reduction in seven subjects receiving angiotensin infusion. Bradley and Parker (39), also using the ballistocardiograph, found an average of 25 per cent reduction in four experiments using single injections and an average fall of less than 10 per cent in three experiments using constant infusions of angiotensin. Courmand et al (40), using both ballistocardiographic and direct Fick measurements, found a small, but consistent fall in the cardiac output of human subjects after a single injection of angiotonin. Finnerty (41), using the dye-dilution method, found a striking fall in the cardiac output in one of three subjects receiving angiotensin by infusion.

In the present experiments, the cardiac output of animals receiving angiotensin infusions was not significantly different from the colony mean. It was, however, a little smaller than that of control animals which received saline infusion. However, the differences were not significant statistically and did not appear to be biologically important. Our data certainly do not justify the conclusion that there is a fall in cardiac output after angiotensin, but neither should they
be interpreted to indicate that there is not. What is clear is that any reduction in cardiac output produced by angiotensin, if it exists, is not of major importance. The conclusions, which are expressed in the following discussion, are not altered qualitatively by the assumption that the cardiac output is reduced.

The changes in blood pressure produced by angiotensin infusion have, in the past, been found to be relatively independent of either the infusion rate or the duration of the infusion (\( \text{H2, H3} \)). This is not true of single injections.

In our experiments, essentially the same finding was made. Infusion of 0.05 \( \mu \text{g/kg/min.} \) had no effect on blood pressure. All larger infusions had approximately the same pressor effect. The effect was maximal after 3\( \frac{1}{2} \) minutes of any infusion level. The mean blood pressure (113 mm. Hg) in these animals was elevated approximately 45 per cent.

The total peripheral resistance was increased almost precisely as much as the mean arterial pressure (44 per cent), the expected result in the face of an unchanged cardiac output. This finding is consistent with those of all others who have studied the peripheral vascular effects of angiotensin.
MYOCARDIAL BLOOD FLOW AND RESISTANCE

In perfused hearts and heart-lung preparations, angiotensin has always been found to increase the coronary vascular resistance and diminish coronary blood flow. In the Langendorff preparation, Meier et al. (114) found angiotensin to have a weak vasoconstrictor activity associated with a weak positive inotropic effect. Lorber (145) noted a marked fall in the coronary flow after angiotensin injection in the perfused cat heart. He concluded that the reduced flow was secondary to coronary vasoconstriction. Elek and Katz (146), using a Langendorff preparation, noted a fall in coronary flow on two occasions, an increase in flow on four occasions, and no effect in one. Lorber and Visscher (147) noted a marked coronary vasoconstriction in isolated cat hearts after angiotensin.

Hill and Andrus (28) noted a fall of 45 per cent in coronary flow in the cat heart-lung preparation with increased amplitude of contractions. Middleton and Wiggers (36) noted myocardial depression in their heart-lung preparations after angiotensin, and suggested that this might be secondary to a fall in coronary flow, although this was not directly measured.

In intact animals, the effects of angiotensin on coronary flow and resistance are much less consistent. Maxwell et al. (29), using the nitrous oxide method in intact dogs, found a 30 per cent rise in
coronary vascular resistance, a 30 per cent rise in blood pressure, and no change in coronary blood flow after angiotensin infusion. Potgeiter et al (31, 32), also using the nitrous oxide method, noted a marked increase (90 per cent) in coronary blood flow of intact dogs during angiotensin infusion. They felt that the increase in coronary flow was secondary to increased anaerobic cardiac metabolism. Barer (33) noted a slight fall in coronary blood flow in cats. She used the electromagnetic flowmeter in her studies.

Some of the discrepancies in the results in intact animals may have depended on species' variability, difference in response to various dose levels, or on the limitations of the methods employed. The nitrous oxide method, for example, may have yielded misleading results because of the possibility of faulty equilibration in the experimental conditions (148).

In the present experiments, after the infusion of angiotensin, both the myocardial flow fraction and flow value are increased. Both are increased approximately as much as the blood pressure, and the myocardial vascular resistance remains essentially constant.

The conclusion that angiotensin exerts no specific effect on the coronary vessels is apparently indicated by our results. However, when it is recalled that angiotensin increases the work of the heart by increasing arterial pressure, the possibility must be considered that a vasoconstrictor effect due to a specific action of angiotensin is overcome by a metabolic effect due to the increased requirements made of the heart muscle. This view is supported by the findings of Potgieter et al, as previously discussed. Results in perfused preparations and
The author would like to acknowledge the help of Mr. Francesco Arcidiacono and Mrs. Opal Banner for their invaluable assistance during the experiments. I would also like to acknowledge the valuable assistance and teaching of Dr. Leo A. Sapirstein, who has helped to guide me in experimental physiology throughout my years in the College of Medicine of The Ohio State University. His methods of teaching and of experimentation have been one of the prime motivations in stirring my interest to experimental physiology and medicine. He is one of the few teachers with the rare talent of being able to combine excellence in teaching with excellence in research. The years spent under his supervision have been very rewarding in the degree of knowledge attained and research accomplished.
heart-lung preparations, in which the work of the heart is not altered, suggest that angiotensin is, indeed, a coronary constrictor but that this effect is overridden by other mechanisms in the intact animal. This particular action requires further investigation.
BRONCHIAL BLOOD FLOW AND RESISTANCE

The results on bronchial flow and resistance follow the same pattern as the coronary flow values, i.e., bronchial flow is increased in proportion to the increase in pressure following angiotensin. We are not familiar with any previous investigations on the effects of angiotensin on bronchial circulation.
RENAL BLOOD FLOW AND RESISTANCE

Of all the regional cardiovascular effects of angiotensin, the one most widely agreed upon is the increase in renal vascular resistance and decrease in renal blood flow. In 1940, Corcoran and Page (49) found diodrast clearance to be decreased by infusions of angiotensin. Inulin clearance rose. They concluded that angiotensin exerted its primary effect by vasoconstriction of the efferent arterioles. A year later, the same authors obtained similar results with single injections (50). Similar results and conclusions were reported later by Herrick et al (51). Hughes-Jones et al (52) observed a fall in diodrast clearance in the rabbit after angiotensin. Finnerty et al (41) found the renal blood flow to be reduced in man by angiotensin. Diodrast clearance in man was reduced by angiotensin according to Peart (53). In all of these experiments, the filtration fraction was consistently elevated suggesting that the efferent arteriole was predominantly affected. Results with the electromagnetic flowmeter lead to the same conclusions as the clearance determinations. Assali and Westersten (42) found the renal blood flow to be reduced in dogs and sheep infused with angiotensin despite the rise in mean arterial blood pressure. Barer (33) found the same in the cat. Many other authors have obtained similar results in many species and at almost every level of angiotensin infusion.
Our findings are consistent with those in the literature. Beginning with the angiotensin infusions of 0.1 ug/kg/min., there was regularly observed a 40 per cent reduction in renal blood flow and a 100 to 200 per cent increase in renal vascular resistance. The smallest infusion, 0.05 ug/kg/min., failed to influence renal vascular resistance, but also failed to influence the arterial pressure. This was also consistent with the findings of others (42) who have noted that an increased renal vascular resistance and a rise in blood pressure occur together.
SKIN FLOW AND RESISTANCE

Abell and Page (54), using direct observation in the ear of the rabbit, noted vasoconstriction without a decline in the rate of perfusion after angiotonin. Landis et al (55), using kidney extracts presumably containing renin, noted no change in the skin temperature of the rabbit ear. Corcoran and Page (49), using angiotonin, found no change in the skin temperature of the dog. Using the plethysmographic method, Schales et al (34) noted a marked fall in hand blood flow, but no effect on forearm flow. This was also noted by Wilkins and Duncan (38). These results may be interpreted to indicate that cutaneous vessels constrict, muscular vessels being unaffected by angiotensin. Bock (56), using a thermal conductivity method, found relatively no change in skin flow during intravenous infusion of angiotensin, although skin flow was markedly reduced when angiotensin was given intraarterially.
SPLANCHNIC BLOOD FLOW AND RESISTANCE

Information regarding the effects of angiotensin on the splanchnic bed is surprisingly sparse. Abell and Page (57) noted a narrowing of the mesenteric vessels after angiotensin, but believed that the volume flow of blood was unaffected. Barer (33), using electromagnetic flowmeters, found mesenteric blood flow to be reduced between the duodenum and the terminal colon after single injections of 0.2 to 0.4 ug. in the cat.

In our experiments, intestinal blood flow was unaffected by angiotensin at any infusion rate. Increased pressure was balanced precisely by increased resistance. Although hepatic resistance appeared to fluctuate in an unpredictable fashion, flow values on the whole seemed to indicate some diversion of blood through the hepatic artery.

The spleen responded to angiotensin infusion with an increase in vascular resistance rather less in degree than the increase in blood pressure. The splenic blood flow appeared to be favored in the redistribution of flow which occurred during the infusion of angiotensin. This response is quite different from that seen after other pressor agents as epinephrine, norepinephrine, and pitressin.
CARCASS BLOOD FLOW AND RESISTANCE

In these experiments, the carcass represented all tissues remaining after removal of the internal organs and the skin. It may be assumed that its behavior is descriptive of that of skeletal muscle, which makes up most of its mass.

An increase in skeletal muscular blood flow after angiotensin infusions was found by Bock (56), using a thermal method. Assali and Westersten (42) noted a marked rise in femoral blood flows after single injection and a more delayed rise with constant infusions of angiotensin. On the other hand, Meier (44) found the blood flow of the hind limbs of animals to be decreased, as did Wilkins and Duncan (38). Schales (31) found forearm blood flow to be unchanged in man after angiotensin. Some of these discrepancies may be due to a possible phasic nature of the response. Herrick et al (51) noted an initial transient fall in femoral blood flow, which was then followed by a more pronounced and prolonged increase. This phenomenon was also noted by Barer (33).

In our experiments, the carcass blood flow was slightly, but consistently increased, especially at the higher infusion levels. At these doses, the vascular resistance of the carcass increased about 3.4 per cent, while the blood pressure at the same time increased 4.5 per cent. Our observations, which were made only after prolonged infusions
and were presumably in the hyperemic phase, tend to confirm those of Bock. The possibility that muscle blood flow behaves in a phasic fashion during angiotensin infusion would not be revealed by these experiments.
ADRENAL BLOOD FLOW AND RESISTANCE

The flow fraction to the adrenal was increased at all infusion levels except the largest, but was significant only at the 0.05 ug/kg/min. level. Adrenal blood flow rose correspondingly. The adrenal resistance fell quite significantly at the lowest dose level of 0.05 ug/kg/min., was increased at the highest dose level of 0.5 ug/kg/min., and remained relatively unchanged at the intermediate doses employed. There was, therefore, diversion of blood to the adrenal gland in animals receiving angiotensin infusion. We were unable to find any previous studies in the literature on adrenal blood flow in the presence of angiotensin.

There is much evidence indicating a relationship between aldosterone, its site of production, (the zona glomerulosa) and angiotensin and the production site of renin, (the juxtaglomerular apparatus of the kidney) (58-61). It is tempting to speculate that the increased blood flow is secondary to increased metabolic activity or requirements of the gland due to either increased release or production of aldosterone or both. The reduction in adrenal blood flow at the largest dose level may have been associated with overriding of this response of the adrenal vasculature by the nonspecific vasoconstrictor effects of angiotensin.
CEREBRAL BLOOD FLOW AND RESISTANCE

To our knowledge, there have been no previous reports on the effects of angiotensin on the cerebral blood flow of either man or animals.

In the present experiments, the indicator-fractionation technique was applied to the measurement of cerebral blood flow using iodo$^{131}$ antipyrine as the indicator. In the control animal, a constant cerebral content of the label during the first minute shows that the extraction ratios of brain and body for the label differ from each other by an inconsiderable amount. Animals infused with angiotensin, on the other hand, show declining cerebral label content with time during the first minute.

The import of this decline is that the cerebral extraction ratio for iodoantipyrine is less than that of the body; consequently, the cerebral blood flow fraction is underestimated by the fraction of the label found in the brain after several recirculations have occurred. However, it is possible to describe the cerebral content of the label at a time when both brain and body have the same extraction ratio, i.e., 1.00, either by making observations very early (before venous removal of label has occurred), or by extrapolating later observations of cerebral label content to that time. The latter presupposes that label removal by the venous blood will proceed in an orderly and regular manner, so that the extrapolation is a legitimate one.
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Actually, it has not yet been established in what fashion the observed data should be extrapolated. The simplest and most convenient type of extrapolation, i.e., a linear one, was carried out in the estimation of cerebral flow fraction. Theoretical considerations suggest that a more correct extrapolation would be an exponential one; this would make the extrapolated values higher than those obtained by the linear extrapolation. The values obtained for flow fraction by linear extrapolation probably are less therefore than the true ones.

The time to which the data should be extrapolated is also not certain. It seems probable that the best estimate can be obtained by extrapolating the data to the time when arterial delivery is complete; this is probably about 7 seconds after injection. In our extrapolation carried out here, 10 seconds was taken as the correct time; this choice of time also would serve to make the estimated flow fraction smaller than the true one.

When calculated in this manner, the cerebral flow fraction was some 70 per cent greater than the control values. This is a minimum estimate. The cerebral vascular resistance fell at infusion rates of 0.05 to 0.1 ug/kg/min. At the higher infusion rates, the cerebral resistance appeared to approach normal values, but it must be recognized that a falsely low estimate of flow might have resulted in a falsely high estimate of resistance. It seems advisable to consider at the present time that cerebral vascular resistance is either decreased or unchanged by angiotensin at any dose level employed.

The most remarkable finding was made at the infusion rate of 0.05 ug/kg/min. At this infusion level, there is no measurable change in
blood pressure. Nevertheless, the cerebral blood flow was increased 60 per cent. Correspondingly, the cerebral vascular resistance was reduced 37 per cent.

The results suggest that angiotensin acts at vasodilator and vasoconstrictor sites in the cerebral vasculature. This may be a direct effect of the agent, or angiotensin may cause an increase in cerebral metabolism with a resultant vasodilation of the cerebral vessels. The smaller response at the higher infusion levels could represent increased vasoconstrictor activity tending to offset the metabolic effect.

Whatever the mechanism, it appears from these results that the cerebral blood flow of the pentobarbital anesthetized rat is increased by angiotensin at the infusion rates employed.
CONCLUSIONS

The hemodynamic effects demonstrated in these experiments are in large part consistent with those expected or measured in arterial hypertension, but in this case it is the differences which bear importance. All the similarities which tend to support the idea that angiotensin is the causative agent of hypertension are weakened by a single observation of a difference in the effects of angiotensin and hypertension. Such differences have been observed.

Nowhere in the literature is it reported that cerebral blood flow is increased or cerebral resistance decreased. In fact, the opposite is usually reported. There is no known statement in the literature that there is a cardiac hyperemia (increased flow per unit mass). Most investigators have reported a constant flow per unit mass.

These differences would appear to be sufficient to rule out the possibility that angiotensin is the causative agent in hypertension. This conclusion should be made however, only with this reservation: our experiments were based on infusions of short duration. Perhaps over a course of time the animal will accommodate to this pressor agent in such a manner as to mimic more precisely the hemodynamic changes of the hypertensive animal. Furthermore, the data show a diversion of blood to the adrenal gland, particularly at the lowest infusion rate of 0.05 ug/kg/min., where the greatest effect is also
noted in the brain. The hemodynamic response of these two organs when contrasted with the other tissues is quite marked. One might ask whether the cerebral response is due to the change in adrenal activity, as manifested by the increase in perfusion rate, or vice versa. The relationship between angiotensin and aldosterone also becomes an issue. It is known that angiotensin increases the rate of secretion of aldosterone. It is also known that aldosterone has a marked positive inotropic effect on the heart (62, 63) as well as marked tension producing qualities on vascular smooth muscle in the presence of levarterenol (64). The question is implied as to whether aldosterone has a specific hypertrophic action on the heart. Such a mechanism, however, is not necessary. Increased arterial pressure of itself is sufficient to cause the hypertrophy by inducing an extra work load upon the heart, greater than that induced by increasing the venous return. Furthermore, at no time was an increase noted in the minute output, in fact, if any change was apparent, it might be a small decrease. Studies on the effects of angiotensin show a moderate to marked bradycardia with a concomitant decrease in cardiac output sufficient to be secondary to the fall in the pulse rate, or no change in minute output, implying a small rise in the stroke output. Reznak (65) has noted a marked decrease in the cardiac output in rats made hypertensive by aortic ring constriction. The decrease in minute output was accompanied by a moderate tachycardia implying a fall in stroke volume. The changes were first seen within one to two hours after application of the constriction and persisted for approximately one week after which time hypertrophy of the heart was noticeable, reaching
a maximum after three weeks. However, cardiac output and pulse rate were normal after the first week following the insult. Although the methods of inducing hypertension are quite distinct, the fact that both right and left atrial pressures are increased in this preparation cannot be denied. Such an increase should cause increased secretion of aldosterone with its subsequent actions.

At the present time it is not possible to predict the effect of aldosterone in these experiments. What should be clear, however, is that the results are due to the angiotensin infusion in the intact, though anesthetized animal. It would appear to us that our experiments do not substantiate the theory that renal hypertension is secondary to angiotensin. It is hoped that further experimentation on hypertensive animals as well as aldosterone-infused animals will help to clarify this issue.


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I, Morris Jack Mandel, was born on December 2, 1929, in Cleveland, Ohio, to William and Sarah Mandel. My primary education was accomplished in the Cleveland Public School system. In 1948, I graduated from Glenville High School, Cleveland, Ohio. My undergraduate training was completed in 1952 when I received the degree of Bachelor of Science from Kent State University, Kent, Ohio. In 1952, I became a research assistant and then a research fellow in the Department of Physiology at The Ohio State University. The following year I entered the College of Medicine at the same university and continued with my graduate studies in physiology for an additional year.

Although I was not enrolled in graduate school during the remaining period of my medical training, my graduate studies did not cease. I continued in my research activities under the supervision of Dr. Leo A. Sapirstein during the school year and between quarters. During this time I was able to complete eight journal publications. Upon graduation from the College of Medicine I was co-winner of the Borden Award in undergraduate medicine with a fellow classmate, Nicholas Bellios.

My internship was done at Mount Sinai Hospital of Cleveland, Ohio. On completion of my internship, I was awarded a fellowship with the Central Ohio Heart Association and completed another publication during this period at The Ohio State University. After three months, I had
to cancel my fellowship due to special problems at home. Having returned to Cleveland, I undertook one year of training in Internal Medicine at the Highland View Cuyahoga County Hospital and another year at the Cleveland Veterans Administration Hospital. During this time I married the former Carol Ann Bonem and at present have one child. At the end of the two year training program, I entered the United States Air Force and was assigned to Wright-Patterson Air Force Base in the Aerospace Medical Research Laboratories. My assignment is concerned with the effects of vibration on man in terms of both physiological changes and physical endurance.

During my military tenure, I have continued with my graduate studies at The Ohio State University under the supervision of Dr. Leo A. Sapirstein and have completed two publications while in the Department of Physiology, upon one of which my dissertation is based. In addition to my training at The Ohio State University, I have taken a six week course in Radioisotope Techniques at the Oak Ridge Institute of Nuclear Studies in Oak Ridge, Tennessee. Upon completion of my military obligation in October, 1963, I plan to enter into academic medicine and hope to continue with animal studies as well as human studies where the latter may be applicable. This is to be combined with medical teaching of students and trainees in internal medicine.
EFFECT OF ANGIOTENSIN INFUSION
ON REGIONAL BLOOD FLOW AND REGIONAL VASCULAR RESISTANCE IN THE RAT
INTRODUCTION

The study of renal hypertension as a distinct physiological entity received a major impetus from the work of Richard Bright (1), who, in 1836, published his findings relating cardiac hypertrophy to renal disease. Sixty years elapsed before further major information was added to the problem. In 1898 Tigerstedt and Bergman (2) showed that saline extracts of the kidney, when injected into rabbits had a marked pressor effect. To the impure substance responsible for the effect, they gave the name renin. Following another interval of several years, many different investigators tried without success to produce hypertension in the laboratory animal. In 1934, Goldblatt and co-workers (3) demonstrated that bilateral renal ischemia produced with an arterial clamp consistently gave rise to hypertension in the dog. Their work ushered in the modern era of hypertension research. Soon afterwards, many different methods were developed in other laboratories for producing renal hypertension. Page et al (4) were able to produce hypertension in their laboratory by wrapping the decapsulated kidneys in cellophane or silk, thus producing a moderate to severe perinephritis. Grollman (5), by use of a bilateral figure of eight tie on the kidneys of young rats or a unilateral figure of eight tie with a contralateral nephrectomy, noted a fair degree of success in the production of hypertension in the rat.
Although other methods for the production of hypertension have also been developed, the three mentioned above are the most popular and have met with the most success. It should be stated, however, that a different approach (nonoperative) has also met with success. Sapirstein et al (6) showed that the substitution of hypertonic solutions of sodium chloride for drinking water would cause an arterial hypertension in the rat associated with cardiac and renal hypertrophy, but no other gross pathology. Desoxycorticosterone acetate given to rats will also cause arterial hypertension after several weeks to months, but when hypertonic sodium chloride solutions are substituted for the drinking water, the hypertension is enhanced in both time and severity (7). Griffith (8) and Olson and Deane (9) showed that choline deficient diets in weanling rats caused a hemorrhagic tubular degeneration in the kidneys, which healed by the sixteenth day following the initiation of the diet. Potassium deficiency caused a tubular necrosis with vacuolization and dilation of the tubules (10). This information was used by Grollman and White (11) as well as Hartroft and Best (12) who found that such diets fed to weanling rats produced severe hypertension in those animals that survived to maturity. Examination of the kidneys at this time revealed no evidence of damage. Indeed, such animals show no alteration in renal hemodynamics in contrast to animals made hypertensive by other means (9, 13). Recently, Grollman has gone even further (14). Rats at various stages of pregnancy were given diets known to be nephrotoxic to weanlings;
all the offspring which survived one year were hypertensive. Although this admittedly may result from other factors, the diets undoubtedly played some nephrotoxic role in the fetus.

Armed with the knowledge that renal ischemia caused hypertension and that saline extracts of kidneys caused a pressor response, many investigators attempted to establish a causal relationship. Page et al (15) and Braun-Menendez et al (16), working independently, showed that renin was an enzyme and not the pressor substance itself. This led to their now classical description of the renin-angiotensin system. (Although angiotensin was formerly referred to as angiotonin by Page et al, and hypertensin by Braun-Menendez et al, the compromise of angiotensin was resolved in 1958 in a meeting of the two principal investigators (17).

It has been suggested that following renal ischemia, renin is released by the kidney into the blood stream, where it converts hypertensinogen, an alpha-2-globulin, into angiotensin, the pressor substance. With the passage of time, renin continues to be released at a low rate sufficient to maintain the chronic hypertensive state.

Although there is much data to support the concept of the renin-angiotensin system as the precipitating cause of renal hypertension, there is marked controversy as to whether this mechanism plays any role in the maintenance of the chronic state.

Although the condition of hypertension predominantly affects the cardiovascular system, studies on the effects of hypertension on this system have been primarily confined to the cardiac output, renal blood flow, cerebral blood flow, and coronary blood flow. While renal blood