Angiotensin in Blood and Lymph Following Reduction in Renal Arterial Perfusion Pressure in Dogs

By Sandford L. Skinner, M.D., James W. McCubbin, M.D., and Irvine H. Page, M.D.

Lever and Peart have described the appearance of renin in lymph after partial occlusion of a renal artery. The importance of this route of release compared with direct release into renal vein blood is unknown. This has prompted us to compare the amount of pressor material entering the circulation in lymph with that in blood during procedures that reduce renal perfusion pressure.

Methods

Fasting adult mongrel dogs weighing 9 to 15 kg were anesthetized with morphine sulfate (2 mg/kg subcutaneously) and sodium pentobarbital (15 mg/kg intravenously) or with sodium pentobarbital alone (30 mg/kg intravenously). In some experiments the vagus-sympathetic-depressor trunks were cut. Intermittent positive pressure respiration was used unless otherwise stated. Arterial pressure was recorded from a femoral artery by a mercury or a strain gauge manometer. Five per cent dextrose in saline was given by slow intravenous drip in all experiments.

In 17 dogs, one kidney was removed and a Goldblatt clamp applied loosely to the contralateral renal artery. Both procedures were performed retroperitoneally through flank incisions. In 8 of these 17 dogs, the thoracic duct was cannulated in the neck and lymph allowed to drain externally. Renal vein blood was obtained in 10 dogs by introducing a polyethylene catheter (outside diameter 2 mm) into the renal vein on the nephrectomized side, passing it across the inferior vena cava into the contralateral renal vein and manipulating it into the renal hilum. Arterial blood was obtained through a polyethylene catheter from a femoral artery. Blood samples (3 to 5 ml) were collected in polyethylene test tubes before, during, and after 45- to 60-minute periods of renal artery constriction accomplished by tightening the Goldblatt clamp until pulsations distal to the clamp were reduced. In this group of experiments there was no objective measure of the reduction in renal perfusion pressure. All blood samples were heparinized and centrifuged at room temperature for 5 minutes at 5,000 rev/min; the plasma was frozen for subsequent assay of pressor activity. The time between sampling and refrigeration ranged between 10 and 15 minutes. Care was exercised to avoid hemolysis and accompanying release of angiotensinase.

An alternative procedure for reducing renal arterial pressure was employed in eight dogs. An 8 Fr. Foley catheter was introduced into the aorta from a femoral artery so that the balloon came to lie just above the renal arteries. Its position was checked by palpation through a flank incision. Inflation of the balloon enabled the pressure in the distal aorta to be controlled at 60 mm Hg or below. Polyethylene catheters were placed in the renal vein via the left testicular or ovarian vein. Arterial blood was sampled from a catheter in the left axillary artery. The thoracic duct was cannulated in the neck and lymph allowed to drain externally. Heparinized blood was handled in the same manner and both lymph and plasma were kept frozen until subsequent assays for pressor activity. In any experiment, the time from sampling to refrigeration was kept as constant as possible for both plasma and lymph. This varied from 10 to 15 minutes in different experiments. In order...
ANGIOTENSIN IN BLOOD AND LYMPH

To facilitate thoracic duct lymph flow, which depends upon normal respiratory changes in intrathoracic pressure, these dogs were anesthetized with sodium pentobarbital alone and allowed to breathe spontaneously.

Hog renin was infused intravenously into seven dogs at varying rates over 30-minute periods while arterial blood and thoracic duct lymph were collected. Two of these dogs were bilaterally nephrectomized through retroperitoneal flank incisions one hour prior to the renin infusion. Samples of blood and lymph were treated in the manner described above.

ASSAY OF PRESSOR ACTIVITY IN PLASMA AND LYMPH

Samples of plasma and lymph were assayed for pressor activity in ganglion-blocked, 150 to 250 g rats, anesthetized with intraperitoneal sodium pentobarbital (6 mg/100 g) containing atropine sulfate (0.2 mg/100 g). Subcutaneous pentolinium tartrate (4 mg/100 g) was used for ganglion blockade. Arterial pressure was recorded from the carotid artery with a mercury manometer or a strain gauge manometer. Samples of lymph and plasma (0.1 to 0.4 ml) were injected into the external jugular vein at the rate of 0.8 ml/min. All samples were kept cold throughout the assay but were brought to room temperature immediately prior to injection. Angiotensin (aspartyl-isoleucyl octapeptide) was used at frequent intervals to determine the sensitivity of the preparation.

Plasma and lymph were assayed without further incubation beyond that which occurred during sampling and centrifugation at room temperature. In some samples, the proteins were precipitated as follows: two volumes of plasma or lymph were diluted with one volume of saline and boiled for 10 minutes. The precipitate was removed by centrifugation. In three experiments, recovery of added angiotensin was essentially complete; one of these experiments is illustrated in figure 2.

Drugs employed were norepinephrine bitartrate, tyramine hydrochloride, serotonin creatinine sulfate, piperoxan hydrochloride, and 2-bromo-L-lysergic acid diethylamide bitartrate. Except for

FIGURE 1

Arteriovenous difference in plasma pressor activity before, during, and after 60 minutes of renal artery constriction (discussion in text). Upper trace is dog's mean femoral arterial pressure; numbers refer to time of collection of peripheral arterial and renal venous plasma. Lower trace is assay of plasma pressor activity using rat's carotid arterial pressure. A: angiotensin, 1 ng (0.1 ml); R: hog renin (0.15 ml); a: arterial plasma (0.1 ml); v: renal venous plasma (0.1 ml).
RESULTS

UNILATERAL RENAL ARTERY CONstriction

Tightening of the Goldblatt clamp on the remaining renal artery caused a gradual rise in blood pressure (10 to 30 mm Hg) which reached a steady level in 15 to 45 minutes in 16 of 17 dogs. After 45 minutes of renal artery constriction, there was no significant difference in the pressor responses of the eight dogs in which lymph was drained externally (15 ± 9 mm Hg) from those of the nine control dogs (16 ± 7 mm Hg). The elevated pressure was maintained as long as the clamp was applied (up to two hours) and, in both groups of dogs, pressure returned to the control level within 15 to 30 minutes after loosening the clamp.

Figure 1 illustrates a typical experiment in which assay of renal venous and peripheral arterial plasma revealed the appearance of pressor material, first in the renal vein and later in arterial blood, during a 60-minute period of arterial constriction. Samples of arterial and renal vein plasma were collected simultaneously at the times indicated. The assay of plasma pressor activity in the rat is shown in the bottom tracing where corresponding arterial and venous samples (0.1 ml) have been injected in sequence. It can be seen that after only seven minutes of arterial constriction, renal venous plasma showed considerable pressor activity while there was no change in either the pressor activity of arterial blood or the blood pressure of the dog. There was a gradual increase in pressor activity of arterial plasma but the renal venous blood contained more pressor activity than arterial blood throughout the period of arterial constriction. Within 15 minutes after release of the clamp the pressor material had disappeared from both arterial and renal venous blood. This pattern of response was seen in each of 10 experiments. In every instance the pressor response to the active plasma resembled the shape of control angiotensin responses superimposed on the small, more sustained response to plasma itself; it

norepinephrine, dosages are expressed in terms of the salt.
**Figure 4**

Pressure in subclavian artery and in lower aorta before, during, and after inflation of balloon placed just above the renal arteries. Dog, 12.5 kg.

**Table 1**

<table>
<thead>
<tr>
<th>No.</th>
<th>Injected volume ml</th>
<th>Control plasma mm Hg</th>
<th>Active plasma mm Hg</th>
<th>Difference mm Hg</th>
<th>Approximate angiotensin in 1 ml active plasma nanograms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.4</td>
<td>10</td>
<td>28</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>14</td>
<td>40</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>3</td>
<td>12</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
<td>4</td>
<td>20</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>3</td>
<td>15</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>0.2</td>
<td>7</td>
<td>24</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>0.1</td>
<td>5</td>
<td>12</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>0.1</td>
<td>10</td>
<td>29</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>0.2</td>
<td>4</td>
<td>15</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>10</td>
<td>0.2</td>
<td>4</td>
<td>32</td>
<td>28</td>
<td>17</td>
</tr>
</tbody>
</table>

*Approximate angiotensin in 1 ml active plasma nanograms.*
FIGURE 5A
Dog, 14.8 kg. Effect of reduction in renal perfusion pressure on flow rate and pressor activity of thoracic duct lymph. The shaded motif represents pressure at the renal arteries and the time base across the motif applies to the diagram above this line. Upper: Lymph flow (ml/min) at 15-minute intervals. Middle: Pressor responses in rat to 0.4 ml lymph samples; responses traced from assay below. Lower: Rat carotid artery pressure. Assay of pressor activity in lymph. A: angiotensin, 1.7 ng; B: hog renin; N: norepinephrine, 10 ng; B: piperoxan hydrochloride, 1.5 mg. 1, 3, 5, 7, 9: Lymph samples (0.4 ml) collected at times indicated above.

340

...did not have the characteristics of the responses to hog renin (fig. 1).

In two experiments, a 30-minute incubation at 37°C caused significant increase in pressor activity of renal venous plasma collected during renal artery constriction, but had no such effect on the control plasmas.

The difference in pressor activity between control plasma samples and samples taken during renal artery constriction was not affected by treating the rat with piperoxan hydrochloride (0.75 mg/100 g iv) or with 2-brom-ß-lysergic acid diethylamide bitartrate (0.05 mg/100 g iv) in amounts sufficient to reduce, or abolish, comparable pressor responses to norepinephrine and serotonin respectively. The pressor material in active samples was not affected by heat precipitation...
of protein but was destroyed by chymotrypsin as shown in figure 2.

These findings suggest that the pressor material is a polypeptide. Evidence that the active material is angiotensin was obtained from pressor responses to tyramine during renal artery constriction. It is known that the pressor responses to tyramine are augmented during intravenous infusion of angiotensin or renin. Figure 3 shows one of five similar experiments in which response to tyramine is augmented during renal artery constriction, while the pressor effect of norepinephrine is little affected. Manipulation of the clamp at the commencement of renal artery constriction caused a sudden rise in pressure, which, however, was soon followed by the usual slow rise and then, after loosening the clamp, a gradual fall to a steady level. Response to tyramine remained slightly augmented 30 minutes after release of the clamp. Augmentation was found to persist for 45 minutes in some experiments.

Since it is highly probable that the difference in pressor activity between control and active plasma is accounted for by angiotensin, it was possible to calculate (by paired assay with known amounts of angiotensin) that under the conditions of these experiments approximately 4 to 20 ng (nanograms = 10^-9 g) of angiotensin were present in each ml of renal vein plasma. The data are summarized in table 1.

**AORTIC BALLOON EXPERIMENTS**

The plan of these experiments is illustrated in figure 4. As a result of balloon inflation, distal aortic pressure could be maintained between 30 and 60 mm Hg for 30 minutes. Following the initial slight increase in subclavian arterial pressure as a result of balloon inflation in the abdominal aorta, there was slow rise in pressure of time sequence and degree similar to that seen during unilateral renal artery constriction. Following balloon deflation, pressure returned to control levels during 15 to 30 minutes. This pattern was seen in all eight experiments. Samples of blood and thoracic duct lymph were collected before, during, and after balloon inflation and assayed for pressor activity in rats.

Figures 5A, 5B, and 5C illustrate results from one of the eight experiments, all of which gave similar results. In these diagrams the shaded motif represents the sequence of blood pressure change in the distal aorta (i.e., at the renal arteries) and the time base across
Nephrectomized dog, 13.6 kg. Effect of intravenous infusion of hog renin on pressor activity of arterial plasma and thoracic duct lymph. Upper: Mean femoral artery pressure before, during, and after 30-minute infusion of renin. Middle: Pressor activity of 0.2 ml plasma and lymph collected at the times indicated. Responses traced from assay below. Lower: Rat carotid artery pressure. Assay of pressor activity in 0.2 ml lymph and plasma. A: angiotensin, 1 ng.

FIGURE 6

the motif applies to the diagram above this line only. In figure 5A, lymph flow, measured at 15-minute intervals, was increased during the period of balloon inflation but returned to control levels afterwards. This was a consistent finding in all experiments, the increase varying from 50% to 100%. The pressor responses in rats to 0.4 ml of these 15-minute lymph samples are illustrated in sequence. These responses have been traced from one or more assays, one of which is at the bottom of the diagram. Pressor material did not appear in thoracic duct lymph until 15 minutes following balloon deflation; it was present for another 60 minutes. Because of the number of samples in this experiment, the entire assay could not be performed on one rat and the time course of the appearance of pressor material is a composite diagram of the results on two rats. Whenever this was necessary, differences in sensitivity between rats were carefully determined.

The rat assay in figure 5A illustrated a frequent finding. The control lymph sample produced a definite pressor response on two occasions early in the assay; this response to control samples diminished subsequently. Smaller volumes (0.1 to 0.2 ml) used in most assays also showed this effect, which could not be accounted for either by a change in sensitivity of the rat or by the nonspecific effect of injected volume. Although the early response to sample 1 was similar to a control renin response, it was not due to renin, since incubation of these control lymph samples did not increase their pressor activity, and since the addition of a comparable amount of renin to control lymph, followed by incubation for 15 minutes at room temperature, resulted in the appearance of considerable angiotensin-like activity. The rat rapidly becomes insensitive to this unknown substance and it does not interfere with the assay. Plasma behaves similarly (fig. 5C). The latter part of the lymph assay demonstrated that the pressor difference between samples 1 and 7 is little affected by piperoxan, while the pressor effect of norepinephrine is abolished. This increased pressor activity of lymph was not destroyed by heat precipitation of protein but was eliminated by incubation with chymotrypsin.

Figure 5B demonstrated the occurrence of pressor activity in renal vein blood in the same experiment as figure 5A and collected at the same time as the lymph samples. It is apparent that pressor activity appeared earlier in the renal vein than in thoracic duct and that the pressor activity in renal venous plasma is slightly greater than that in lymph. This can be seen by comparison with standard angiotensin responses and by direct comparison of blood and lymph in the same rat. The prolonged response to sample 3 of renal vein blood (fig. 5B) is unexplained, but since it was not seen in other experiments, it probably has no importance. Also, the persistence of pressor activity up to 30 minutes after balloon inflation was not seen in other experiments, in all of which it had returned to
ANGIOTENSIN IN BLOOD AND LYMPH

control levels by this time. Again, the use of piperoxan demonstrated that this pressor material is not norepinephrine.

Figure 5C illustrates the occurrence of pressor activity in peripheral arterial blood during the same experiment discussed in figures 5A and 5B. Pressor activity, similar to that in renal venous plasma, appeared in arterial plasma within 15 minutes of reduction in renal perfusion pressure and persisted for at least 30 minutes after balloon deflation. The pressor assays were conducted in the same manner as those in figures 5A and 5B.

In two of this group of eight experiments, an increase in pressor activity of lymph could not be detected, despite the usual increase in renal venous and arterial plasma. Since, in the other six experiments, the pressor activity of lymph rose to a similar degree as that of arterial blood, the possibility had to be excluded that the presence of angiotensin in lymph was simply a result of that which was already in arterial plasma, filtering slowly into lymph. To investigate this possibility, the following experiments were performed.

RENIN INFUSION

In seven experiments, hog renin was infused intravenously over 30-minute periods in amounts that raised the blood pressure to, or above, the levels seen during the balloon experiments. Thoracic duct lymph and peripheral arterial blood were collected before, during, and after renin infusion and assayed for pressor activity. Figure 6 illustrates that a renin infusion producing a 40-mm Hg rise in arterial blood pressure resulted in appearance of considerable angiotensin-like activity in arterial blood without corresponding change in lymph. It was only when renin was infused in much greater amounts that any change in pressor activity of lymph occurred, and even then it was much less than in plasma. Similar results were obtained in both normal and nephrectomized dogs.

Discussion

These experiments are in accord with those of Lever and Peart in that renal pressor material appears in lymph in increased amounts soon after reduction of renal perfusion pressure, but they are at variance in that large amounts of angiotensin were found in renal vein blood. Under the circumstances of our experiments, the latter route is the primary one by which renin is released into the circulation.

When renin is secreted into blood or lymph it produces angiotensin I, from which angiotensin II is formed, and this, in turn, is continuously inactivated by angiotensinase. Clearly, a method that measures only total pressor activity cannot be used to analyze this complex enzyme system. Our purpose, instead, was to obtain clear evidence for the presence of this system in renal vein blood and lymph when renal perfusion pressure is reduced, and to establish with reasonable certainty the primary route of release.

Braun-Menéndez and Fasciolo showed that grafting an ischemic kidney to the circulation of a nephrectomized dog caused a renin-like response. There followed a long period during which many mutually contradicting papers appeared, a consequence in large part of lack of reliable and sensitive assay methods. Recent investigations have yielded more satisfactory results. Helmer has been able consistently to show the presence of renin, or renin-like material, in blood of patients with known renal hypertension, and Genest reports a positive correlation between presence of angiotensin in blood and rate of excretion of aldosterone. The literature in this field has been reviewed recently.

In the present experiments, a slow rise in blood pressure occurred within as few as five minutes following partial constriction of a renal artery and was accompanied by appearance of a pressor substance in renal vein plasma that persisted for the duration of the rise in pressure. The origin of the pressor material appears to be the kidney since activity appeared later in arterial blood and in much smaller amounts than in renal vein blood. The greater amount of pressor material in renal vein plasma persisted throughout the systemic pressor response following reduction of renal perfusion pressure.
We believe that the pressor material demonstrable in renal vein blood is angiotensin formed from renin released by the kidney. By pharmacological means, the pressor material was shown not to be norepinephrine or serotonin. Characteristic of a polypeptide, it was not destroyed by heat-precipitation of protein and was inactivated by chymotrypsin. The rise in blood pressure following renal artery constriction was accompanied by enhanced pressor responsiveness to tyramine like that which occurs on intravenous infusion of angiotensin. The shape of the curve of pressor response to active plasma was like that to angiotensin rather than to renin. Renin was probably present, however, since incubation of the active, but not control, plasma increased its angiotensin-like pressor activity.

The results indicate that renin is released mainly by way of renal vein blood rather than into renal lymph. External drainage of the thoracic duct lymph, which includes renal lymph, did not have a significant effect on the magnitude or duration of the systemic pressor response following renal artery constriction and did not prevent the appearance of angiotensin in circulating blood. To avoid possible obstruction of lymph drainage by the clamp on the renal artery, a balloon was placed in the abdominal aorta above the origin of the renal arteries and inflated so as to reduce renal perfusion pressure. Renal plasma flow, although severely reduced at perfusion pressures of 30 to 60 mm Hg, would not be nearly as low as thoracic duct lymph flow (0.2 to 0.5 ml/min), and yet more angiotensin was found per unit volume of renal vein plasma than lymph. In these experiments it required 30 minutes or more for lymph to pass from the kidney to the neck as evidenced by the appearance of pressor material (fig. 5A). Because of this interval, renin in lymph was in contact with substrate for a longer period than was the case in renal vein plasma. The incubation time for plasma was increased to 30 minutes in some experiments, and there was further increase in the difference in pressor activity between plasma and lymph. These results would be misleading if there were a deficiency of renin-substrate in lymph, or if increased amounts of angiotensinase occur there. Friedman et al. demonstrated that comparable amounts of renin-substrate occur in lymph and plasma of dogs and that the quantity of angiotensinase in lymph is considerably less than that in plasma.

We conclude that a short period of reduced renal arterial perfusion pressure causes release of considerable amounts of renin into renal venous blood, and much smaller amounts into renal lymph.

Summary

Systemic pressor responses occurred consistently 5 to 15 minutes following reduction of renal perfusion pressure and were accompanied by the appearance of pressor material in renal vein blood. The pressor material is probably angiotensin formed from renin released by the kidney, since its activity was not affected by protein precipitation, was eliminated by incubation with chymotrypsin, and since it caused enhanced pressor responses to tyramine, all of which are characteristic of angiotensin. Activity was unaffected by pharmacologic blockade of response to norepinephrine and serotonin. Its renal origin is indicated by the greater amount of pressor activity in renal venous than in arterial blood throughout the systemic pressor responses. Smaller amounts of pressor material, also with properties of angiotensin, were found in thoracic duct lymph. They did not contribute materially to the systemic pressor response since external drainage of all lymph did not modify the response, and since the time course of the response correlated with an increase in pressor activity in renal vein blood but not in lymph. The late appearance of angiotensin in thoracic duct lymph is probably due to renin secretion by the kidney into renal lymph rather than transfer from circulating blood; activity did not appear in lymph when renin was infused intravenously in amounts that caused rises in systemic pressure comparable to those produced by reduction of renal perfusion pressure.
ANGIOTENSIN IN BLOOD AND LYMPH

The results indicate that during short periods of reduced renal perfusion pressure, renin is released in larger amounts in blood than in lymph.

Acknowledgment

We are grateful to Miss Katherine Fowler and Mr. Robert Binker for expert technical assistance.

References

Angiotensin in Blood and Lymph Following Reduction in Renal Arterial Perfusion Pressure in Dogs

SANDFORD L. SKINNER, JAMES W. MCCUBBIN and IRVINE H. PAGE

doi: 10.1161/01.RES.13.4.336

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1963 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/13/4/336