Hepatic Clearance of Renin in Canine Experimental Models for Low- and High-Output Heart Failure

By Edward G. Schneider, James O. Davis, Charles A. Robb, and John S. Baumber

ABSTRACT

Hepatic blood flow, hepatic extraction of renin, and hepatic clearance of renin were compared in normal conscious dogs, in dogs with constriction of the thoracic inferior vena cava (as a model of low-output heart failure), and in dogs with an aortic-caval fistula (high-output heart failure). Both "failure" preparations showed marked sodium retention. Renin was measured by methods described previously and expressed as nanograms of angiotensin produced during incubation per milliliter of plasma. Hepatic plasma flow was determined by the sodium sulfobromophthalein method. The dogs with low-output heart failure had a marked reduction in hepatic plasma flow, while those with high-output failure showed no significant change in this flow. Plasma renin was significantly elevated in both models of heart failure. Dogs with low-output failure had an increase (P<.05) in hepatic renin extraction from a normal value of 19.8% to 33.4%, while those with high-output failure showed a decrease in hepatic renin extraction to 11.9% (P<.01). The hepatic clearance of renin in low-output failure (104 ml/min) was the same as the average normal value of 104 ml/min, while clearance of renin by the liver in high-output failure was reduced to 51 ml/min (P<.001). The data provide evidence that the decreased metabolism of renin contributes substantially to the increased plasma level of renin in experimental high-output heart failure.

ADDITIONAL KEY WORDS hepatic extraction of renin sodium retention thoracic inferior vena caval constriction aortic-caval fistula plasma renin hepatic blood flow chronic venous congestion

In congestive heart failure, in decompensated cirrhosis of the liver, and in their experimental counterparts, elevated plasma renin levels (1-7) have been causally related to increased aldosterone secretion. The exact mechanisms which lead to an elevated plasma renin have not been clearly defined. Haecoix, et al. (8) have demonstrated in anesthetized dogs that significant extraction or inactivation of renin is brought about by the liver. In view of the hepatic congestion associated with right heart failure, it was decided to investigate the role of the hepatic metabolism of renin in the elevated plasma renin levels found in experimental heart failure. Hepatic blood flow, hepatic renin extraction, and the hepatic clearance of renin were measured in normal conscious dogs and in conscious dogs with either constriction of the thoracic inferior vena cava or an aortic-caval fistula.

Constriction of the thoracic inferior vena cava was used to produce a model of low-output heart failure. Although such animals are not in true congestive heart failure, it has been demonstrated that the salt and...
water metabolism is very similar if not identical to that observed in authentic low-output failure (3). Dogs with an aortic-caval fistula were used as a model of high-output heart failure.

Methods

For chronic studies in conscious dogs, a polyvinyl chloride catheter (French no. 8) was placed under sterile conditions into a large hepatic vein in 17 female mongrel dogs (16 to 25 kg). The catheter was brought out through a puncture wound in the abdominal midline area and was protected by a heavy cloth jacket. The catheter was periodically flushed with heparin to prevent clotting. The thoracic inferior vena cava was constricted in five dogs and a large (12 to 14 mm long) aortic-caval fistula was placed 2 to 2.5 inches below the kidneys in six other animals. The dogs were placed in metabolic balance cages to measure daily sodium excretion. All animals received 65 mEq of sodium daily in the diet and water ad libitum. The animals were studied after they had recovered from the surgical procedure (5 to 7 days) and when the dogs used as models of heart failure had demonstrated the appropriate syndrome of salt and water metabolism and, in the fistula preparations, signs of cardiac failure.

EXPERIMENTAL PROCEDURES

Eighteen hours after eating, conscious dogs were brought into the laboratory. A saphenous vein was intubated with a polyethylene catheter (P.E. 50) to administer the sodium sulfobromophthalein (BSP) priming solution (2 mg/kg) and to infuse a maintenance solution (.08 mg BSP/kg/min). A blood sample for BSP analysis was collected before the priming solution of BSP was given. After an equilibration period, samples of hepatic and jugular vein blood (10 ml) were collected simultaneously for renin and BSP analysis at 60, 90, and 120 minutes. The blood samples were collected in test tubes treated with 10 mg disodium-ethylene diamine tetraacetate acid and placed in ice. Inferior vena caval pressure was measured with a water manometer immediately after the experiment. To determine if an arterial-venous plasma renin difference existed, samples were collected simultaneously from the femoral artery and the jugular vein of six normal animals.

ANALYTICAL METHODS

The BSP concentration of unhemolyzed plasma was determined according to the method of Gaebler (9).

Renin was determined by the method of Wathen et al. (10) as modified by Schneider et al. (11). The method consisted of dialyzing 2 ml of plasma against 0.01M EDTA for 6 hours followed by a second dialysis against 0.02M PO4 at pH 5.3 for 18 hours. After dialysis, 0.05 ml of a 1:100 disisopropyl-fluorophosphate solution and 0.052 ml of saturated NaCl were added to the plasma. The dialyzed plasma was then incubated at 37°C for 3 hours in graduated test tubes. The samples were heat inactivated to stop the reaction, the volume was adjusted to 4 ml with a buffered saline solution, and the supernatant fluid was frozen and stored for bioassay in the rat (12). Synthetic angiotensin II (Hypertensin, CIBA) was used as the standard. All samples were assayed in triplicate, and the results were expressed as nanograms of angiotensin produced per milliter of plasma.

Calculations

1. Total hepatic plasma flow (THPF) (ml/min) =
   infusion rate of BSP (mg/min)
   / (jugular vein BSP − hepatic vein BSP (mg/ml))

2. Renin extraction (%) =
   (jugular vein renin − hepatic vein renin)
   / jugular vein renin

3. Hepatic extraction of renin (HEr) (ng/min) = THPF × (jugular vein renin − hepatic vein renin)

4. Hepatic clearance of renin (HCr) (ml/min) = HEr / jugular vein renin

Results

HEPATIC CLEARANCE OF RENIN IN NORMAL DOGS

The results obtained on six normal dogs are presented in Table 1. The extraction ratio for BSP was 41.2 ± 4.9% with a hepatic plasma flow of 27.3 ± 1.4 ml/min/kg and a hepatic blood flow of 45.8 ± 2.9 ml/min/kg. No difference was found in the plasma renin concentration between samples from the femoral artery and the jugular vein collected simultaneously in six dogs; the mean values and standard errors of the means were 4.9 ± 0.9 ng/ml and 4.8 ± 1.0 ng/ml, respectively, for femoral artery and jugular vein samples. The percent hepatic extraction of renin was 19.8 ± 2.9, which is in agreement with the finding of Haecox et al. (8) of a 25% hepatic extraction of renin in anesthetized
The rate of hepatic extraction of renin was $510\pm 74 \text{ ng/min}$, while the hepatic clearance of renin was $104.8\pm 15.5 \text{ ml/min}$.

**HEPATIC CLEARANCE OF RENIN IN DOGS WITH THORACIC INFERIOR VENA CAVA CONSTRICTION**

In all dogs the rate of renal sodium excretion was less than 2 mEq/day and all had ascites formation. Venous pressure below the constriction was elevated from a control level of 30 to 60 mm to 175 to 330 mm H$_2$O. Duplicate sets of measurements were made a few days apart on dogs 4 and 7; determinations of venous pressure and sodium excretion revealed no changes during the interval between studies. There was a significant decrease in hepatic plasma flow and hepatic blood flow with no change in the percent BSP extraction (Table 2). Plasma renin was markedly increased in all dogs with vena cava constriction from an average normal value of 5.0 to 36.4 ng/ml. The hepatic extraction of renin increased from a control value of $510\pm 74 \text{ ng/min}$ to $3670\pm 918 \text{ ng/min}$. In spite of the decreased plasma flow, the hepatic clearance of renin was unchanged, because renin extraction by the liver increased from a control value of $19.8\pm 2.9\%$ to $33.4\pm 6.8\%$.

**HEPATIC CLEARANCE OF RENIN IN DOGS WITH HIGH-OUTPUT HEART FAILURE**

In the dogs with high-output failure, renal sodium excretion was less than 5 mEq/day, and peripheral edema or ascites or both were present. Central venous pressure was elevated from 30 to 60 up to 82 to 203 mm H$_2$O, while arterial pressure below the shunt ranged from 60 to 80 mm Hg. Four of the six animals were studied on two occasions; renal sodium excretion and venous pressure were essentially the same during the two sets of measurements. Five of the six dogs died of pulmonary edema 1 to 5 weeks after completion of the experimental study. The sixth dog was killed after two months with massive ascites.

The data obtained on the dogs with high-output heart failure are presented in Table 2. No change occurred in the percent of BSP extracted by the liver. Although hepatic plas-
**TABLE 2**

**Hepatic Clearance of Renin in Dogs**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Wt (kg)</th>
<th>Hct</th>
<th>BSP extraction (%)</th>
<th>Hepatic flow (ml/min/kg)</th>
<th>Jugular vein renin (ng/ml)</th>
<th>Hepatic vein renin (ng/ml)</th>
<th>Renin extraction (%)</th>
<th>Hepatic extraction of renin (ng/min)</th>
<th>Hepatic clearance of renin (ml/min)</th>
<th>Venous pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>25</td>
<td>40</td>
<td>34.0</td>
<td>20.4</td>
<td>34.0</td>
<td>14.5</td>
<td>9.8</td>
<td>32.4</td>
<td>2340</td>
<td>159.0</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
<td>39</td>
<td>16.3</td>
<td>17.2</td>
<td>27.7</td>
<td>29.8</td>
<td>23.2</td>
<td>22.1</td>
<td>2480</td>
<td>80.0</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>38</td>
<td>29.2</td>
<td>17.5</td>
<td>20.5</td>
<td>14.3</td>
<td>9.2</td>
<td>35.6</td>
<td>1610</td>
<td>112.0</td>
</tr>
<tr>
<td>7</td>
<td>19</td>
<td>50</td>
<td>54.8</td>
<td>9.0</td>
<td>18.0</td>
<td>52.2</td>
<td>20.6</td>
<td>61.0</td>
<td>5400</td>
<td>103.0</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>62</td>
<td>62.5</td>
<td>9.5</td>
<td>25.6</td>
<td>71.2</td>
<td>32.0</td>
<td>55.0</td>
<td>7530</td>
<td>106.0</td>
</tr>
<tr>
<td>MEAN</td>
<td>21</td>
<td>42</td>
<td>33.4</td>
<td>16.3</td>
<td>27.1</td>
<td>36.4</td>
<td>23.7</td>
<td>33.4</td>
<td>3670</td>
<td>104.5</td>
</tr>
<tr>
<td>±SEM</td>
<td>4</td>
<td></td>
<td>6.8</td>
<td>1.9</td>
<td>2.1</td>
<td>11.1</td>
<td>7.8</td>
<td>6.8</td>
<td>918</td>
<td>15.9</td>
</tr>
<tr>
<td>P*</td>
<td>&gt; .4</td>
<td>&gt; .2</td>
<td>&lt; .002</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .05</td>
<td>&lt; .01</td>
<td>&gt; .5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Thoracic Vena Cava Constriction**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Wt (kg)</th>
<th>Hct</th>
<th>BSP extraction (%)</th>
<th>Hepatic flow (ml/min/kg)</th>
<th>Jugular vein renin (ng/ml)</th>
<th>Hepatic vein renin (ng/ml)</th>
<th>Renin extraction (%)</th>
<th>Hepatic extraction of renin (ng/min)</th>
<th>Hepatic clearance of renin (ml/min)</th>
<th>Venous pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>25</td>
<td>.38</td>
<td>50.9</td>
<td>20.1</td>
<td>32.4</td>
<td>12.0</td>
<td>11.1</td>
<td>7.5</td>
<td>440</td>
<td>36.9</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>.26</td>
<td>27.1</td>
<td>30.3</td>
<td>41.0</td>
<td>19.6</td>
<td>17.0</td>
<td>13.3</td>
<td>1440</td>
<td>73.6</td>
</tr>
<tr>
<td>11</td>
<td>19</td>
<td>.18</td>
<td>34.5</td>
<td>31.3</td>
<td>38.4</td>
<td>6.9</td>
<td>6.4</td>
<td>7.3</td>
<td>200</td>
<td>42.0</td>
</tr>
<tr>
<td>12</td>
<td>21</td>
<td>.37</td>
<td>15.8</td>
<td>22.3</td>
<td>35.2</td>
<td>10.4</td>
<td>8.8</td>
<td>15.4</td>
<td>740</td>
<td>71.5</td>
</tr>
<tr>
<td>13</td>
<td>21</td>
<td>.37</td>
<td>20.3</td>
<td>17.9</td>
<td>28.3</td>
<td>14.8</td>
<td>12.6</td>
<td>14.9</td>
<td>820</td>
<td>55.3</td>
</tr>
<tr>
<td>14</td>
<td>22</td>
<td>.40</td>
<td>38.0</td>
<td>20.6</td>
<td>34.4</td>
<td>14.1</td>
<td>11.7</td>
<td>17.0</td>
<td>1080</td>
<td>76.3</td>
</tr>
<tr>
<td>MEAN</td>
<td>21</td>
<td>.35</td>
<td>36.4</td>
<td>21.0</td>
<td>31.9</td>
<td>13.0</td>
<td>11.5</td>
<td>11.9</td>
<td>620</td>
<td>51.3</td>
</tr>
<tr>
<td>±SEM</td>
<td>1</td>
<td>.03</td>
<td>5.8</td>
<td>3.0</td>
<td>4.0</td>
<td>1.8</td>
<td>1.7</td>
<td>1.9</td>
<td>112</td>
<td>9.3</td>
</tr>
<tr>
<td>P*</td>
<td>&gt; .1</td>
<td>&gt; .2</td>
<td>&gt; .1</td>
<td>&lt; .02</td>
<td>&lt; .005</td>
<td>&lt; .005</td>
<td>&lt; .01</td>
<td>&gt; .2</td>
<td>&lt; .001</td>
<td></td>
</tr>
</tbody>
</table>

**High-Output Heart Failure**

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Wt (kg)</th>
<th>Hct</th>
<th>BSP extraction (%)</th>
<th>Hepatic flow (ml/min/kg)</th>
<th>Jugular vein renin (ng/ml)</th>
<th>Hepatic vein renin (ng/ml)</th>
<th>Renin extraction (%)</th>
<th>Hepatic extraction of renin (ng/min)</th>
<th>Hepatic clearance of renin (ml/min)</th>
<th>Venous pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>25</td>
<td>.38</td>
<td>50.9</td>
<td>20.1</td>
<td>32.4</td>
<td>12.0</td>
<td>11.1</td>
<td>7.5</td>
<td>440</td>
<td>36.9</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>.26</td>
<td>27.1</td>
<td>30.3</td>
<td>41.0</td>
<td>19.6</td>
<td>17.0</td>
<td>13.3</td>
<td>1440</td>
<td>73.6</td>
</tr>
<tr>
<td>11</td>
<td>19</td>
<td>.18</td>
<td>34.5</td>
<td>31.3</td>
<td>38.4</td>
<td>6.9</td>
<td>6.4</td>
<td>7.3</td>
<td>200</td>
<td>42.0</td>
</tr>
<tr>
<td>12</td>
<td>21</td>
<td>.37</td>
<td>15.8</td>
<td>22.3</td>
<td>35.2</td>
<td>10.4</td>
<td>8.8</td>
<td>15.4</td>
<td>740</td>
<td>71.5</td>
</tr>
<tr>
<td>13</td>
<td>21</td>
<td>.37</td>
<td>20.3</td>
<td>17.9</td>
<td>28.3</td>
<td>14.8</td>
<td>12.6</td>
<td>14.9</td>
<td>820</td>
<td>55.3</td>
</tr>
<tr>
<td>14</td>
<td>22</td>
<td>.40</td>
<td>38.0</td>
<td>20.6</td>
<td>34.4</td>
<td>14.1</td>
<td>11.7</td>
<td>17.0</td>
<td>1080</td>
<td>76.3</td>
</tr>
</tbody>
</table>

*Probability that the mean differs from the value obtained for normal dogs. Student's t-test was used and the degree of freedom was based on the number of dogs studied.
ma flow was lower but not significantly, the fall in hepatic blood flow was significant at the 2% level; hematocrit decreased from 41% to 35%. Although plasma flow was not significantly reduced for the group, four of the six dogs with a fistula (9, 11, 13 and 14) did show values considerably below the average normal value. Plasma renin was higher in dogs with an A-V fistula than in control animals \((P < .005)\) but the increase in plasma renin from 5.0 to 13.0 ng/ml in the dogs with a fistula was considerably less \((P < .001)\) than the marked increase seen in dogs with caval constriction. The percent extraction of renin by the liver was reduced \((P < .01)\) in the dogs with an A-V fistula, but the rate of renin extraction by the liver was unchanged \((P > .2)\). The hepatic clearance of renin was consistently reduced in the dogs with an A-V fistula \((P < .001)\).

**Discussion**

The levels of hepatic blood flow and plasma flow and the percent of BSP extraction found in normal dogs in this study are in excellent agreement with the values reported in the literature for conscious dogs \((13-15)\). Selkurt \((16)\) has shown that the BSP method of determining hepatic blood flow agrees closely with the values found by direct determination. The decrease in hepatic plasma flow and blood flow in dogs with caval constriction and the decreased hepatic blood flow in dogs with an A-V fistula does not appear to be associated with a methodological error, since BSP extraction was not changed and mixing of hepatic vein blood with caval blood would cause an over-estimation, not under-estimation, of hepatic blood flow. From the studies of aldosterone metabolism and the hepatic extraction of aldosterone, it has also been concluded that hepatic plasma flow is decreased in dogs with caval constriction. It was found that the percent aldosterone extracted by the liver was essentially 100% in both normal dogs and dogs with caval constriction \((17)\) and that metabolism occurred primarily in the liver. Consequently, in accordance with the Fick principle, aldosterone metabolism could have decreased only as a result of a decreased hepatic plasma flow. Also, no change in the rate of disappearance of aldosterone from plasma occurred in two dogs with A-V fistula \((17)\), indicating no significant change in hepatic plasma flow; again, aldosterone extraction by the liver was approximately complete. Apparently, therefore, decreased hepatic plasma flow is not an essential feature of high-output heart failure produced by an A-V fistula. In fact, two of the six dogs with A-V fistula in the present study had normal hepatic plasma flow.

Since the value for hepatic renin clearance is based on two experimental determinants, a large normal variation might be expected. However, duplicate experiments run on six of the dogs revealed that the variance from the mean of the two determinations was 11 ± 4% with a range from 3 to 27%. The reason for this low variance is that multiple samples were used to determine the hepatic BSP differences and the assay was done in triplicate on three pairs of jugular-hepatic vein samples for the difference in renin across the liver. Statistical analysis of the data indicated that real differences were present between groups of animals.

The hepatic clearance of renin is a measure of the rate of renin inactivation by the liver. Under conditions of a constant rate of renin secretion, a decrease in the clearance of renin by the liver indicates that the plasma level of renin is higher than normal. Thus, a decrease in renin clearance means that altered liver function plays a role in the maintenance of an elevated plasma level of renin.

The importance of the hepatic clearance of renin in the overall inactivation or metabolism of renin, or both, by the entire organism is not known. However, the finding of Houssay, et al. \((19)\) that hepatectomy decreased the rate of plasma renin disappearance suggests that the liver does play an important role. Haecox et al. \((8)\) found that the hepatic extraction of renin in dogs, within the limitation of their methods, was equal to the rate at which they infused renin. Christlieb et al.
(20) have shown that a significant hepatic extraction of renin occurs in human subjects. Furthermore, Schneider et al. (11) have recently found that the metabolic clearance rate of renin from arterial plasma calculated from the plasma disappearance curve in anesthetized, nephrectomized dogs agrees with the present value obtained for the hepatic clearance of renin in normal conscious dogs. The lack of evidence that other organs or tissues such as the spleen, pancreas, gastrointestinal tract (5), kidney (5, 21), and plasma (personal observation) participate in the inactivation of renin is consistent with other evidence that the liver is the major if not only site of significant renin inactivation.

The importance of an elevated plasma renin level in dogs with thoracic caval constriction has been reviewed by Davis (3). Higgins et al. (2) presented data indicating that there was an increase in renin secretion in dogs with caval constriction. Since no change in hepatic renin clearance was shown with caval constriction, the data indicate that the elevated plasma renin levels are due only to an increased renin secretion. Indeed, the liver extracts renin more efficiently under these conditions, as indicated by the increased percent renin extraction and the increased absolute amount of renin extracted by the liver from normal values of 510 ng/min to 3670 ng/min. This increase in renin extraction might be a consequence of hepatic venous congestion in the dogs with caval constriction. Thus, a decrease in liver blood flow per unit of hepatic blood volume could conceivably provide a favorable time dependency for more complete renin extraction. Using an exogenous renin infusion, Haecox et al. (8) found anesthetized dogs to have the same percent renin extraction by the liver at plasma renin levels three to five times that of control dogs. This indicated that the normal hepatic inactivating system is capable of handling high renin levels.

Davis et al. (5) demonstrated that the hypersecretion of aldosterone was associated with retention of sodium in dogs with a large (12 to 14 mm) A-V fistula. Recently, Johnston et al. (1) found an elevated plasma renin level in only three of five dogs with A-V fistula and marked sodium retention. The present finding of an elevated plasma renin level in all but one dog (14) with a fistula and a change significant for the group at the .005 level adds support to the concept that the renin-angiotensin system is important in hypersecretion of aldosterone in high-output heart failure. The statistically significant reduction in the percent hepatic extraction of renin for the group of dogs with an A-V fistula occurred concurrent with a decreased hepatic plasma flow in four of the six dogs and resulted in the decrease of hepatic renin clearance to about half of the normal value for the group of animals. Also, in light of the low magnitude of the plasma renin elevation in high-output failure, a significant portion of the plasma renin elevation can be attributed to a decrease in the hepatic clearance of renin. Indeed, an average increase of 130% in plasma renin was associated with a 51% reduction in the clearance of renin by the liver. In contrast to the marked hypergranularity of the juxtaglomerular cells in dogs with caval constriction (22), an index of renin secretion, the lack of a similar hypergranulation in dogs with an A-V fistula (1) further implicates the importance of a decreased renin clearance by the liver in maintaining the elevated plasma renin found in experimental high-output heart failure.

The mechanism responsible for the reduction in the hepatic clearance of renin in high-output failure cannot be deduced from this study. Neither chronic venous congestion nor a decrease in hepatic blood flow, as demonstrated by the dogs with caval constriction, appears to produce the depression of hepatic renin clearance. Apparently some other undefined biochemical or cardiovascular factor is present in high-output heart failure. Regardless of the nature of this factor, a decreased rate of renin clearance by the liver contributes substantially to the high plasma renin in high-output failure.

Circulation Research, Volume XXIV, February 1969
Acknowledgments

The authors wish to thank Mr. Michael Atherton and Mr. John Haas for their help with the chemical analyses. The sodium sulfobromophthalein (BSP) was kindly supplied by Hynson, Westcott and Dunning, Inc., of Baltimore, Maryland.

References


Hepatic Clearance of Renin in Canine Experimental Models for Low- and High-Output Heart Failure
EDWARD G. SCHNEIDER, James O. Davis, Charles A. ROBB and John S. Baumber

Circ Res. 1969;24:213-219
doi: 10.1161/01.RES.24.2.213

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1969 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/24/2/213

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/