Arteriolar Wall Thickening in Hypertensive Rats Unrelated to Pressure or Sympathoadrenergic Influences

Walter C. Plunkett and Henry W. Overbeck

We have previously reported that experimental aortic coarctation in rats is accompanied by non-pressure-related increases in the wall-to-lumen ratio (W/L) of cremaster arterioles. To investigate the role of the sympathoadrenergic system in this arteriolar wall thickening, we partially constricted or sham-constricted the abdominal aorta in adrenal-demedullated, 6-week-old rats that had had guanethidine injections to produce peripheral sympathectomy (S rats, n = 17 coarcted, 16 sham-coarcted) and in sham-demedullated, sham-sympathectomized control rats (SS rats, n = 13 coarcted, 15 sham-coarcted). In both SS and S rats with coarctation, tail and femoral arterial and conscious abdominal aortic pressures were not increased but carotid pressures rose by >30% (p<0.01), accompanied by 46–75% increases in cardiac ventricular weight/body weight. In coarcted rats, 4–6 weeks after aortic constriction, compared with sham-coarcted rats, whether S or SS, observation of the cremaster microcirculation revealed increased wall area, wall thickness, and W/L of third- to fifth-order arterioles, both in the resting state and after maximal relaxation with topical nitroprusside. For example, in coarcted S rats wall area after nitroprusside was elevated by 24%, 39%, and 37% in third-, fourth-, and fifth-order arterioles (p<0.01). These findings indicate that arteriolar wall thickening in hypertension may occur independently of intra-arterial pressure or sympathoadrenergic influences. Humoral growth factors may be involved. (Circulation Research 1988;63:937–943)

Increasing evidence suggests an important role for non-pressure-related factors in cardiovascular wall thickening in hypertension. However, the mechanisms of such pressure-independent growth have not been identified. Among other factors, sympathoadrenergic influences have been incriminated. Bevan1 observed that sympathetic denervation impaired proliferation of vascular smooth muscle cells in the ear artery of the growing rabbit, and Hart et al2 observed that sympathetic denervation inhibited the development of cerebral vascular hypertrophy in stroke prone spontaneously hypertensive rats. These reports and others3,4 provide strong evidence for a major trophic role of the sympathetic nervous system.

In animals with coarctation hypertension, intra-arterial pressure distal to the aortic constriction remains normal.5–13 Thus, this model allows the role and nature of the underlying mechanisms involved in the hypertensive process to be studied independently of the effects of intravascular pressure. From pressure-flow studies in this model, we found evidence for an increased structural component of hind limb vascular resistance that persisted after guanethidine ablation of the peripheral sympathoadrenergic system.5 The purpose of the present study was to seek confirmation of this previous finding by direct observation of cremaster arterioles in coarcted rats.

Methods and Materials

Sprague Dawley rats (Zivic-Miller Laboratories, Zelienople, Pennsylvania) were randomly divided into four groups. Newborn male rats received guanethidine sulfate (Ismelin Sulfate, kindly supplied by CIBA-GEIGY), 50 mg/kg/day i.p., 5 days per week for 3 weeks (aged 7–27 days) for a total of 15 injections.14 At 28 days of age these rats underwent bilateral surgical adrenal demedullation.15 These rats were designated "sympathectomized." Control rats, designated "sham-sympathectomized" rats, received equal volumes of saline intraperitoneal 5 days per week at ages 7–27 days and sham adrenal...
demudulation at age 28 days. As in our previous studies,5,11 coarctation hypertension was created in 17 sympathectomized and 13 sham-sympathectomized rats weighing between 140-190 g (approximately 5–6 weeks of age) by placing a partially constricting silver clip (0.813 mm i.d.) around the abdominal aorta above both renal arteries. In 16 sympathectomized and 15 sham-sympathectomized rats, a clip (1.48 mm i.d.) too large to constrict the aorta was similarly placed. These rats served as sham-coarcted normotensive controls. All rats were maintained on standard rat chow (Wayne Lab Blocks; 0.39% Na and 0.96% K) and tap water ad libitum. Weekly tail blood pressures in conscious rats were measured by tail plethysmography (Natsume Tail Manometer System, Tokyo, Japan).

Four to six weeks postoperatively, rats were prepared for study of the cremaster microcirculation. Rats were anesthetized by intraperitoneal injection of chloralose and urethane (1:13.3%)16 and placed on a heated Lucite board. Rectal temperature was maintained at 37° C, and a tracheostomy was performed to maintain a patent airway. The left carotid and left femoral arteries were cannulated for blood pressure measurements. Mean arterial blood pressure was measured with a Statham P23Gb transducer (Gould, Cleveland, Ohio) and Hewlett-Packard Model 8805C pressure amplifier and 7702B recorder (Palo Alto, California).

We prepared the left cremaster muscle for transillumination by methods previously described.13 Briefly, we made a ventral incision in the scrotal sac and carefully teased the cremaster with its testicle free from the associated connective tissue. Using a cautery to control bleeding, we cut the ventral aspect of the cremaster from the caudal pole to the inguinal ring. The testicle and connective tissue were teased free from the cremaster muscle and irrigated with physiological saline solution. The muscle was pulled evenly over a glass-topped pedestal with 35-gauge surgical wire, and a cover glass was placed on top of the muscle. As the excess fluid dripped, a seal spontaneously forms around the edges of the cover slip and allows equilibration of oxygen between the blood and vasculature.

With the rat on the microscope stage, transillumination of the cremaster muscle (remote 50-W halogen light source with variable power supply) allowed us to visualize the microcirculation with the aid of Nikon optics (×20 objectives, ×15 eyepiece, a long-range working condenser) and a television monitoring system (Hitachi CCTV camera, model HV 17LU, and television monitor VM 129U). Before making observations, we inspected the muscle for impaired blood flow, petechiae, and other signs of vessel damage. Due to the age of the animals, some arterioles in both hypertensive and normotensive rats were not adequately visualized; those vessels that did not have clearly visible walls or that did not show vasomotion were not used. When stable vasomotion was reestablished (usually 60–90 minutes after surgery), observations were made at random in either the thin or thick side of the cremaster. The vessels studied were the third-, fourth-, and fifth-order arterioles. Order of arterioles was identified by branching pattern, with the main cremaster artery being the first-order arteriole and each branch being sequentially numbered as has been described.17 We measured the internal diameter, which did not include the arteriolar walls, and an outside diameter, which did include the arteriolar walls and the adventitia. For each vessel we measured internal and outside diameter repeatedly and recorded averaged values.

The cremaster microcirculation was first studied in the resting (normal) state and then after maximal vascular relaxation with nitroprusside (0.2 ml of a 10 mg/ml solution) applied topically. This concentration of nitroprusside is greater than that previously found by Miller et al18 to have a maximal vasodilatory effect; 10 times this concentration produced no further vascular relaxation. Observations were carried out during the subsequent hour. Replication of nitroprusside at 30 minutes and at the end of the hour caused no further relaxation. Each experiment lasted 4–5 hours, and no supplemental anesthesia was necessary.

In each rat we measured internal diameter and outside diameter of four or five different vessels in each of the three orders of arterioles. From the internal diameter and outside diameter measurements, we calculated wall thickness [(OD-ID)/2] = {π(ID/2)}^2/4, and cross-sectional area of the wall [(π(OD/2)^2)−{π(ID/2)^2})], where OD is outside diameter and ID is internal diameter. These individual measurements were averaged for each order arteriole within each rat. The averaged values (n = number of rats) were then subjected to statistical analysis.

Terminally, blood was taken for measurement of hematocrit, plasma sodium and potassium (flame photometry [model 643, Instrumentation Laboratory, Dayton, Ohio]) and creatinine (Creatinine Assay, Sigma Chemical, St. Louis, Missouri). Each rat was necropsied, the cardiac ventricles were weighed, and clip type and placement, as well as general health, were verified.

In a second study, rats were prepared for observations of cremaster arterioles as already described. However, in this study, 50, 150, 250, and 500 ng of norepinephrine (arterenol bitartrate, Sigma), each in a volume of 0.02 ml, was injected into the femoral arterial cannula followed by a 0.5 ml flush and was observed to flow through the vasculature of the cremaster. From change in the average internal diameter, we calculated the percentage of closure.

To further verify that conscious hindquarter pressures were similar in sympathectomized coarcted and sham-coarcted animals, rats were instrumented for conscious blood pressure measurements 1 and 3 weeks after coarctation. Under pentobarbital anesthesia (50 mg/kg), indwelling catheters were placed through the femoral artery into the abdominal aorta.
below the origin of the renal arteries. Twenty-four hours later, blood pressure was measured continuously between 8 AM and 6 PM in the conscious rats, and these pressures were averaged.

Analysis of variance was used to detect differences among the four groups of rats in vascular wall dimensions, body weight, cardiac ventricular weight, ventricular weight corrected for body weight, plasma electrolytes, blood pressure, hematocrit, and plasma creatinine. When analysis of variance indicated differences, we used a multiple comparison procedure (Student-Neuman-Keuls) to assess individual differences. No difference was considered significant unless p<0.05. All values are reported as mean±SEM.

**Results**

Ptosis, enophthalmos, and some mild diarrhea were noted in sympathomctomized rats. At the time the microcirculation was observed, all rats had been gaining weight and appeared healthy, although body weight of sympathomctomized rats ranged 11–14% less than sham-sympathectomized animals (Table 1). Body weights of coarcted rats did not differ from those of the appropriate sham-coarcted rats. Cardiac ventricular weights and ventricular weights corrected for body weight were increased (p<0.01) in coarcted compared with sham-coarcted rats. Terminal hematocrits and creatinines (Table 1), as well as plasma sodium and potassium, did not differ among groups.

Tail blood pressures in conscious coarcted and appropriate sham-coarcted rats did not differ, as we have previously reported.5,6,8-11 Similarly, abdominal aortic pressures measured in conscious instrumented rats at 1 and 3 weeks after clipping did not differ in coarcted and sham-coarcted rats whether sympathectomized or not. In anesthetized rats, carotid blood pressure was significantly elevated by 32–36% (p<0.01) in coarcted compared with the appropriate sham-coarcted rats, although blood pressure was reduced 19–22% by the effects of sympathectomy. Femoral blood pressure was not different between coarcted and appropriate sham-coarcted rats, although, again, sympathectomy reduced blood pressure 21–24%. Topical administration of sodium nitroprusside reduced femoral blood pressure in sham-sympathectomized and sympathectomized rats. After sodium nitroprusside, there were no significant differences between femoral blood pressure in coarcted and appropriate sham-coarcted rats.

Figure 1 represents calculated arteriolar wall areas in the four groups of rats, in both the resting and the totally relaxed (sodium nitroprusside) states. The three major new findings of the present investigation are illustrated in Figure 1. First, coarctation hypertension is associated with significant increases in wall area in third- to fifth-order cremaster arterioles. This is true in both the resting and relaxed states (p<0.01). Increases with coarctation (sham-sympathectomized rats) averaged 61%. Second, in most cases sympathoadrenergic ablation did not prevent these increases in wall area. However, significant interaction between sympathectomy and coarctation indicated that the increases were attenuated, averaging 26%. Third, there was evidence (sham-coarcted rats) that sympathoadrenergic ablation alone results in increases in wall area (three of six comparisons; increases averaging 30%).

Changes in wall-to-lumen ratio (Figure 2) and in wall thickness (Tables 2 and 3) parallel these changes in calculated wall areas. Increases in wall-to-lumen...
ratio ($p<0.01$ in most cases) and in wall thickness ($p<0.01$) accompanied coarctation and were detected in both resting and relaxed arterioles. Again, sympathoadrenergic ablation did not prevent, but may have attenuated (positive interactions in fourth- and fifth-order arterioles), these increases. Again, sympathoadrenergic ablation alone (sham-coarcted rats) resulted in significant increases in both thickness and wall-to-lumen ratio in the smaller-order arterioles. Small and inconsistent decreases in internal diameter and increases in outside diameter with coarctation were also observed (Tables 2 and 3).

The response of third-order arterioles to intraarterial norepinephrine (Table 4) was significantly increased ($p<0.01$) in sympathectomized compared with sham-sympathectomized rats, as would be expected with denervation supersensitivity. However, there was no convincing evidence for differences associated with coarctation.

Discussion
Factors incriminated in the genesis of the non-pressure-related cardiovascular growth observed in hypertension include genetic influences,

humoral factors, growth influences local to the vascular wall or associated cells, and sympathoadrenergic influences. The purpose of the present study was to assess the contribution of sympathoadrenergic influences to the pressure-independent growth we have observed in the normotensive hindquarters of rats with coarctation hypertension. We accomplished sympathoadrenergic ablation with standard procedures that have been validated. Additionally, we verified that such ablation evoked denervation.
TABLE 2. Arteriolar Measurements Before Maximal Vascular Relaxation With Nitroprusside

<table>
<thead>
<tr>
<th>Number of rats</th>
<th>Arterioles:</th>
<th>Sham-Coarcted</th>
<th>Sympathectomy</th>
<th>Coarcted</th>
<th>Sympathectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Internal diameter (μm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd order</td>
<td>20.8 ± 0.6 (55) ab</td>
<td>22.4 ± 1.0 (99) a</td>
<td>19.6 ± 1.0 (55) b</td>
<td>18.9 ± 0.4 (61) b</td>
<td></td>
</tr>
<tr>
<td>4th order</td>
<td>11.7 ± 0.3 (72) ab</td>
<td>12.1 ± 0.5 (80) a</td>
<td>10.5 ± 0.4 (62) ab</td>
<td>10.7 ± 0.3 (80) a</td>
<td></td>
</tr>
<tr>
<td>5th order</td>
<td>7.4 ± 0.4 (76) ab</td>
<td>7.7 ± 0.3 (80) a</td>
<td>6.7 ± 0.3 (53) ab</td>
<td>6.6 ± 0.2 (76) b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outside diameter (μm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd order</td>
<td>27.3 ± 0.7 (55) a</td>
<td>30.0 ± 0.9 (69) a</td>
<td>29.5 ± 1.1 (55) a</td>
<td>27.9 ± 0.6 (61) b</td>
<td></td>
</tr>
<tr>
<td>4th order</td>
<td>16.9 ± 0.3 (72) a</td>
<td>18.4 ± 0.5 (80) a</td>
<td>18.3 ± 0.6 (62) b</td>
<td>18.3 ± 0.3 (80) b</td>
<td></td>
</tr>
<tr>
<td>5th order</td>
<td>11.9 ± 0.3 (76) a</td>
<td>12.6 ± 0.3 (80) ab</td>
<td>13.3 ± 0.5 (53) b</td>
<td>13.4 ± 0.3 (76) b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wall thickness (μm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd order</td>
<td>3.3 ± 0.1 (55) a</td>
<td>3.8 ± 0.1 (69) a</td>
<td>5.0 ± 0.2 (55) b</td>
<td>4.5 ± 0.2 (61) a</td>
<td></td>
</tr>
<tr>
<td>4th order</td>
<td>2.6 ± 0.1 (72) a</td>
<td>3.2 ± 0.1 (80) a</td>
<td>3.9 ± 0.2 (62) b</td>
<td>3.8 ± 0.1 (80) a</td>
<td></td>
</tr>
<tr>
<td>5th order</td>
<td>2.2 ± 0.1 (76) a</td>
<td>2.5 ± 0.1 (80) a</td>
<td>3.3 ± 0.2 (53) b</td>
<td>3.4 ± 0.1 (76) b</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM; values in parentheses are number of vessels observed. The values within a row sharing the same superscript letter are not significantly different (p > 0.05).

viation supersensitivity in the cremaster microcirculation we studied.

The major findings of the present study are the increases we observed in calculated wall area of the cremaster arterioles. In contrast to other measurements (diameters, wall thickness, and wall-to-lumen ratios in resting vessels), wall areas are unaffected by variations in the contractile state of the arteriole because of the incompressibility of tissue. Thus, within a defined arteriolar segment, changes in wall areas reflect changes in the vessel wall tissue mass. Increases in tissue mass, in turn, may indicate cellular hypertrophy or hyperplasia, fibrosis, edema, or a combination. Whatever the nature of the increase in tissue mass, the result would be elevated arteriolar wall-to-lumen ratio with important effects on vascular resistance and responses, as described by Folkow.26 Such effects may be of considerable importance in the disease mechanisms of hypertension.

In the present study of the normotensive vascular bed of the cremaster muscle, we observed that coarctation hypertension is associated with increases averaging about 60% in the wall areas of third- to fifth-order arterioles (outer diameters ranging from 10 to 39 μm) and that sympathoadrenergic ablation does not prevent these increases. Strengthening these conclusions was our associated observation of increases with coarctation of the wall thickness and the wall-to-lumen ratio of these arterioles. These changes remained under conditions of maximal arteriolar relaxation and thus cannot be attributed to artifacts related to differences in contractile state. They also were not prevented by sympathoadrenergic ablation. However, it should be noted that ablation attenuated the increases in all these variables.

These results indicating that non-pressure-related arteriolar wall thickening in coarctation is not prevented by sympathoadrenergic ablation are consistent with our previous observations in this

TABLE 3. Arteriolar Measurements After Maximal Vascular Relaxation With Nitroprusside

<table>
<thead>
<tr>
<th>Number of rats</th>
<th>Arterioles:</th>
<th>Sham-Coarcted</th>
<th>Sympathectomy</th>
<th>Coarcted</th>
<th>Sympathectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Internal diameter (μm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd order</td>
<td>26.7 ± 0.8 (58) a</td>
<td>27.9 ± 0.9 (66) a</td>
<td>29.2 ± 1.1 (47) a</td>
<td>27.5 ± 1.2 (69) a</td>
<td></td>
</tr>
<tr>
<td>4th order</td>
<td>16.8 ± 0.4 (69) a</td>
<td>16.9 ± 0.4 (60) a</td>
<td>16.8 ± 0.9 (61) b</td>
<td>16.8 ± 0.4 (81) a</td>
<td></td>
</tr>
<tr>
<td>5th order</td>
<td>11.3 ± 0.3 (62) ab</td>
<td>11.7 ± 0.2 (71) ab</td>
<td>10.7 ± 0.4 (50) a</td>
<td>11.6 ± 0.3 (75) b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Outside diameter (μm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd order</td>
<td>32.6 ± 0.8 (58) a</td>
<td>34.4 ± 0.9 (66) a</td>
<td>37.3 ± 1.1 (47) a</td>
<td>35.4 ± 1.2 (69) ab</td>
<td></td>
</tr>
<tr>
<td>4th order</td>
<td>21.0 ± 0.4 (69) a</td>
<td>21.7 ± 0.5 (60) ab</td>
<td>23.6 ± 1.1 (61) ab</td>
<td>23.2 ± 0.5 (81) a</td>
<td></td>
</tr>
<tr>
<td>5th order</td>
<td>14.6 ± 0.3 (62) a</td>
<td>15.9 ± 0.3 (71) a</td>
<td>16.0 ± 0.5 (50) b</td>
<td>17.1 ± 0.4 (75) a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wall thickness (μm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd order</td>
<td>3.0 ± 0.1 (58) a</td>
<td>3.2 ± 0.1 (66) a</td>
<td>4.0 ± 0.1 (47) b</td>
<td>3.9 ± 0.1 (69) b</td>
<td></td>
</tr>
<tr>
<td>4th order</td>
<td>2.1 ± 0.1 (69) a</td>
<td>2.4 ± 0.1 (60) a</td>
<td>3.4 ± 0.2 (61) a</td>
<td>3.2 ± 0.1 (81) a</td>
<td></td>
</tr>
<tr>
<td>5th order</td>
<td>1.7 ± 0.1 (62) a</td>
<td>2.1 ± 0.1 (71) a</td>
<td>2.7 ± 0.1 (50) ab</td>
<td>2.7 ± 0.1 (75) a</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SEM; values in parentheses are number of vessels observed. The values within a row sharing the same superscript letter are not significantly different (p > 0.05).
model of hypertension. We had found that similar ablation did not prevent the increases in minimal resistance of the rat hind limb accompanying coarctation hypertension.5

In apparent contrast, Hart et al2 reported that sympathetic denervation inhibited the development of cerebral vascular hypertrophy in stroke-prone spontaneously hypertensive rats. However, in their study wall-to-lumen ratio remained significantly elevated in denervated SHR vessels less than 35 μm diameter, so, regarding the smaller arterioles, the two studies appear consistent. In the present study, effects on larger resistance vessels (up to 300 μm), which may be quite important in the hypertensive process,26 were not investigated.

The present study also provides evidence that sympathectodegenic ablation in normotensive (sham-coarcted) animals is accompanied by thickening of the cremaster arteriolar walls. Similarly, we had previously observed that sympathectomy increased the structural component of hind limb resistance in normotensive rats.5 Our findings are in apparent contrast to Bevan’s observation1 that local sympa-
thetic denervation impaired proliferation of vascular muscle in the ear artery of the growing rabbit. Differences may be related to the vessels observed or the species studied. Our findings also appear to differ from those of Nyborg et al,27 who reported that neonatal sympathectomy of Wistar-Kyoto rats with 6-hydroxydopamine is associated with reduced wall-to-lumen ratio of mesenteric resistance vessels. Differences in the two studies may be related to the central effects of 6-hydroxydopamine or to the vessel studied.

Documentation that the hindquarters remain normotensive in rats with coarctation hypertension is critical for our argument that the wall thickening we observed is not pressure-related. Such verification has been previously reported by us5,6,8–11 and by several other groups.7,12,13 In the present study we also documented that abdominal aortic pressure in instrumented conscious animals with sympatheadrenergic ablation remains at the same level in coarcted and sham-coarcted rats. We have assumed, but not verified, that pressures in the cremaster arterioles reflect those in the abdominal aorta, which seems likely.

Regarding effects of blood flow in coarctation hypertension, Stanek et al13 have recently reported that coarctation is accompanied by small (16%) increases in blood flow in total tissue mass below the site of coarctation. However, hind limb blood flow is unchanged. Effects on cremaster flow were not studied nor have the effects of sympatheoadrenergic ablation on hindquarters (including cremaster) flow been investigated. Thus, it is possible, but we feel it is unlikely, that alterations in cremaster blood flow may account for the wall thickening we observed.

We conclude that a major portion of the non-pressure-related thickening of arteriolar walls that occurs in coarctation hypertension in rats, and probably in other forms of hypertension also,11 cannot be attributed to sympatheoadrenergic influences. Thus, the results of this study provide additional evidence suggesting that humoral factors and/ or growth influences localized to the vascular wall tissues participate in the mechanisms of the abnormal vascular growth that occurs in hypertension.

Acknowledgments

We appreciate the technical assistance of Robert E. Blackmon and Bessie Ingram and the secretarial help of Diana Knotts and April Sandlin.

References

8. Overbeck HW: Cardiovascular hypertrophy and \"waterlogging\" in coarctation hypertension: Role of sympathoadrenergic influences and pressure. *Hypertension* 1979;1:486–492
17. Hutchings PM, Darnell AE: Observations of a decreased number of small arterioles in spontaneously hypertensive rats. *Circ Res* 1974;34 and 35(suppl 1):1-161–1-165

**KEY WORDS** • coarctation hypertension • sympathectomy • microcirculation • cardiovascular hypertrophy • vascular wall-to-lumen ratio
Arteriolar wall thickening in hypertensive rats unrelated to pressure or sympathoadrenergic influences.

W C Plunkett and H W Overbeck

Circ Res. 1988;63:937-943
doi: 10.1161/01.RES.63.5.937

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/63/5/937

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/