A Central Mechanism of Acute Baroreflex Resetting in the Conscious Dog

Wen Tan, Maret J. Panzenbeck, Michael A. Hajdu, and Irving H. Zucker

The role of the central nervous system in the mechanism(s) involved in acute carotid baroreflex resetting was studied in six conscious, chronically instrumented, aortic-denervated dogs. Dogs were prepared for reversible vascular isolation of the carotid sinuses. Acute baroreflex resetting was induced by holding the left carotid sinus pressure (LC_{op}) at a given value for 20 minutes using a pulsatile pressure control system while at the same time keeping the right carotid sinus pressure (RCSP) at a subthreshold level (approximately 40 mm Hg). At the end of the 20 minutes, the LC_{op} was reduced to approximately 20 mm Hg, and a baroreflex (RCSP-mean arterial pressure [MAP]) curve was generated on the right carotid sinus using static-step increases in carotid sinus pressure. At the control LC_{op} of 100 mm Hg, the RCSP-MAP baroreflex had a threshold pressure (P_t) of 86.6±3.1 mm Hg and a set point pressure (P_{sp}) of 104.7±2.5 mm Hg. Increasing LC_{op} to 140 mm Hg for 20 minutes caused these parameters for the right carotid baroreflex to increase. P_t and P_{sp} increased by 18.4±4.0 and 14.2±3.0 mm Hg, respectively (p<0.05). The baroreflex curve, therefore, was shifted upward and to the right. Decreasing LC_{op} to 60 mm Hg caused P_t and P_{sp} to decrease by 24.7±5.0 and 18.1±2 mm Hg, respectively (p<0.05). The baroreflex curve was therefore shifted downward and to the left. The percent of resetting of P_t and P_{sp} was 46±9% and 36±8%, respectively, when LC_{op} was 140 mm Hg. These values were 61±14% and 46±5%, respectively, when the LC_{op} was set to 60 mm Hg. These results show that conditioning of the ipsilateral carotid sinus baroreceptors can induce acute baroreflex resetting from the contralateral, unconditioned carotid sinus. This strongly suggests that the conditioning pressure can cause baroreflex resetting by a central mechanism alone without the need for the receptors themselves to be reset. (Circulation Research 1989;65:63–70)

It is well documented that a change in carotid sinus or arterial pressure for relatively short periods of time can reset the threshold of baroreceptor discharge.\(^1\)–\(^3\) It is believed that this acute baroreceptor-resetting phenomenon will enable the baroreflex to shift its set point as well as its operating range to a higher or lower level during short-term hypertension or hypotension. Previous work from this laboratory has shown that acute baroreflex resetting can also be induced in conscious dogs.\(^4\) Data from some earlier studies have shown that the extent of acute resetting for the baroreflex was greater than for baroreceptor resetting alone. This suggested, therefore, that there may also be involvement of a central component in acute baroreflex resetting, which may result from the interaction of other afferent inputs that may be stimulated during the resetting period.\(^5\) On the other hand, a recent study by Kunze\(^6\) in anesthetized rabbits showed that electrical stimulation of the aortic nerve for 5 minutes reduced the depressor response to subsequent aortic nerve stimulation. This may indicate an influence of baroreceptor afferent input per se on the resetting process. However, to our knowledge, there is no direct physiological evidence showing that conditioning of the baroreceptors with different pressures can cause central resetting of the baroreflex. It is generally believed that baroreceptors account for the major portion of acute baroreflex resetting.\(^7\)

The present study demonstrates that conditioning of carotid baroreceptors alone for 20 minutes in conscious dogs can induce baroreflex resetting through a purely central mechanism. The amount of this resetting is as great as has been previously demonstrated in a study in which both the baroreceptors and the central nervous system (CNS) were involved.\(^4\) These findings suggest a new

---

From the Department of Physiology and Biophysics, University of Nebraska College of Medicine, Omaha, Nebraska.

Supported by National Institutes of Health Grant HL-33359. M.J.P. was supported by a Post-Doctoral Fellowship from the National Institutes of Health (HL-07241).

Address for correspondence: Irving H. Zucker, PhD, Department of Physiology and Biophysics, University of Nebraska College of Medicine, 42nd and Dewey, Omaha, NE 68105.

Received April 5, 1988; accepted December 10, 1988.
concept of baroreflex resetting in which input from baroreceptors can reset its central controller by a substantial degree.

Materials and Methods

Animal Preparation

Aortic denervation. Using sterile technique, six mongrel dogs (20–25 kg) underwent surgery to strip the aortic arch and its major branches. Anesthesia was induced with sodium pentobarbital (30 mg/kg i.v.). The left chest was entered through the fourth intercostal space. The adventitia around the aortic arch and around the vessels emanating from the arch was stripped and painted with a solution of 10% phenol in ethanol. A Tygon catheter was introduced into the descending aorta for the subsequent measurement of aortic pressure. The chest was then closed and evacuated. The dogs were treated with 1.2 million units of Combiotic per day for the next 7 days.

The effectiveness of the aortic denervation was determined after carotid sinus isolation. With the sinuses isolated, arterial pressure was changed with phenylephrine and sodium nitroprusside. Aortic denervation was considered complete if heart rate did not change by more than five beats/min when arterial pressure was changed by at least 50 mm Hg.

Carotid sinus preparation. Figure 1 shows a schematic representation of the experimental preparation. At least 10 days after the thoracotomy, the dogs were prepared for vascular isolation of the carotid sinuses. The technique described by Stephenson and Donald was used. In brief, under pentobarbital anesthesia, the carotid sinuses were exposed through a midline cervical incision. A perivascular occluder was placed on the common carotid artery and on the external carotid. All other branches of the carotid were ligated. A Tygon catheter was placed in the carotid through the thyroid artery. This catheter was cephalad to the common carotid occluder. This preparation eliminated perfusion to the carotid bodies. This was verified in several dogs by the lack of a chemoreflex in response to an intracarotid bolus injection of nicotine. The failure to elicit a chemoreflex was consistent with the data from Stephenson and Donald carried out in a larger group of dogs. All catheters were tunneled beneath the skin and exited the animal from the back of the neck. The carotid catheters were flushed with sodium heparin (1,000 units/ml). The dogs were treated with Combiotic for an additional week. During the recovery period, the dogs were brought into the laboratory and trained to sit quietly on a laboratory table. Approximately 2 weeks after the carotid surgery, the first experiment was performed.

Experimental setup. The occluder and carotid sinus pressure system consisted of two control systems and are shown schematically on the bottom of Figure 1. A constant air pressure of 300 mm Hg...
Central Baroreflex Resetting

The experimental protocol used in these experiments. During the experiment, both sinuses were isolated from the systemic circulation. Right sinus pressure was set at 40 mm Hg, while the left sinus was conditioned with a pulsatile pressure. In the control state the conditioning pressure was 100 mm Hg for 20 minutes; then both left and right sinus pressures were lowered to approximately 20 mm Hg. A baroreflex curve was then generated on the right carotid sinus. The high conditioning pressure was 140 mm Hg and the low conditioning pressure was 60 mm Hg. Each was held for 20 minutes before a right carotid baroreflex curve was drawn. Between each conditioning period, the sinuses were opened to the systemic circulation for a period of 20 minutes.

Carotid sinus pressure–mean arterial pressure curves and resetting indexes. Carotid sinus pressure (CSP)–mean arterial pressure (MAP) curves were generated by pressurizing the right carotid sinus in a stepwise manner. The CSP range was from 20 to 180 mm Hg. Figure 2 shows a representative protocol. Each step was held for about 10 seconds, and the steady-state data was sampled using an on-line computer at the end of that period. It usually took approximately 1.5 minutes to generate a full curve. The sampled data were then fit to a mathematical model as was described by Kent et al. 

Equation 1 indicates the sigmoidal equation that was used to fit the data.

\[
\text{MAP} = \frac{A1}{A1 + \exp(A2 \times (\text{CSP} - A3)) + A4}
\]  

Figure 3 is a representative baroreflex curve fit to the above equation. A1 is the response range, A2 is the average slope of the curve (gain), A3 is also known as \(BP_0\) and is the midpoint of the curve on the x axis, and A4 is the minimum pressure (Pmin), which represents the maximum inhibition of the baroreflex control of arterial pressure. Several other parameters can also be derived from this curve. These are the set point pressure (Psp), threshold pressure (Pth), maximum gain (Gmax), and the CSP where the Gmax exists (GMcsp). Psp is the point where the CSP equals the MAP, that is, where the input of the system equals the output. Since this is an open-loop model, there is no interaction between the input and output signals. Therefore, at equilibrium, there is no baroreflex activation because there is no error signal to be detected. In the steady state, the arterial pressure should be directly related to the set point. The set point not only indicates the state of the CNS controller but also the condition of the baroreceptors, whose thresholds are subject to change. Pth is the CSP that initially activates the baroreflex to decrease arterial pressure. Pth has been determined several different ways. Kent et al. used the third derivative of Equation 1 to indicate threshold. However, we found that the Pth calculated by that method usually was higher than the CSP that existed when an obvious decrease in MAP was seen. This is because the third derivative is the point at which the nonlinear portion of the sigmoid curve changes into a linear portion. Therefore, if the gain of the baroreflex is low around the threshold portion, the shoulder of the curve will be located at a lower portion of the curve, which will make the threshold value higher than it should be. The CSP where the MAP decreases by 5% of the response range as an indication of threshold can be used. However, in this case, Pth will be determined to a great extent by the response range. In the present experiments, we used the CSP where MAP decreased 5% from its maximum value as an index of threshold. Equation 2 was developed to calculate the Pth.
FIGURE 3. A sigmoid regression curve fit to the data obtained from one experiment. The data were fit to Equation 1 in the text as originally described by Kent et al. As, response range; A2, average slope (gain); A3, midpoint pressure; A4, minimum mean arterial pressure. The diagonal line is the line of identity between the carotid sinus pressure and the mean arterial pressure. Pth., threshold pressure; Psp., set point pressure.

Directly from the sigmoid curve parameters that were described by Equation 1.

\[ P_{th} = \frac{\ln \left( \frac{A1}{P_{max} \times 0.95 - A4} - 1 \right)}{A2 + A3} \]  

\[ \text{GM}_{\text{esp}} \] was determined using a graphical technique. The first derivative was drawn over the sigmoid CSP-MAP curve. The pressure at which Gmax occurred was then read off the curves. One advantage of using the CSP at Gmax as an index of resetting is that it is less dependent on the asymmetry of the sigmoid curve. In the current experiments, therefore, Psp, Pth, midpoint pressure (BP50), and GM_{esp} were used as resetting indexes.

To determine the extent of resetting (% resetting), the change in each parameter was compared with the change in the carotid sinus conditioning pressure (CP_{esp}). The following formula was used:

\[ \% \text{resetting} = \frac{\Delta P_{th} \text{ or } P_{sp} \text{ or } BP_{50} \text{ or } \text{GM}_{esp} \times 100}{CP_{esp}} \]  

Protocol and Data Analysis

Figure 2 shows a schematic representation of the experimental protocol. The left carotid sinus was conditioned at a mean pressure of 100, 140, or 60 mm Hg with a pulsatile pressure of 40 mm Hg at a rate of 80 cycles/min. The conditioning period was 20 minutes. The right carotid sinus was held at 40 mm Hg during this period. The change in MAP and heart rate was recorded as a function of time. The pressure at the beginning and the end of the conditioning period has been designated as beginning baseline arterial pressure and end baseline arterial pressure. After 20 minutes, the pulsatile pressure generator was shut off, and both carotid sinus pressures were dropped to about 20 mm Hg. The right intrasinus pressure was then increased in a stepwise manner to generate a right carotid sinus pressure (RCSP)-MAP baroreflex curve. After generation of the curve, all of the occluders were deflated, and the sinuses were reopened to the systemic circulation. The animal was then allowed to rest for about 20 minutes until the MAP and heart rate returned to their resting levels. The next left carotid sinus pressure (LC_{esp}) was then applied and the protocol repeated. The LC_{esp} was applied in random order. The pulsatile pressure was the same for the three LC_{esp}.

Data are presented as mean±1 SEM. Statistical comparisons among groups have been made using a one-way analysis of variance. Individual means were compared using the Ryan-Einot-Gabriel-Welsch multiple F test. A value of \( p<0.05 \) was considered statistically significant.

Results

The MAP of the six dogs at rest with the carotid sinuses open to the circulation was 101±2 mm Hg, and heart rate was 96±7 beats/min. In three dogs, several RCSP-MAP baroreflex curves were generated in succession with the LC_{esp} set at 100 mm Hg for 20 minutes each. There were no significant shifts in these curves and no significant differences in the curve parameters for any of these curves.

Influences of LC_{esp} on RCSP-MAP Baroreflex

In six dogs, the right carotid sinus baroreflex was significantly influenced by changing the conditioning pressure of the left carotid sinus. Figure 4 illustrates a representative experiment in a single dog. In the control panel, the LC_{esp} was set at 100 mm Hg for 20 minutes, then a RCSP-MAP baroreflex was induced by a stepwise increase in RCSP. After a rest period, the RCSP-MAP baroreflex was induced after the LC_{esp} was set at 140 and subsequently at 60
mm Hg. The data from the RCSP-MAP baroreflex were fit to three sigmoid curves and are shown in Figure 5. At high LC$_{csp}$, the curve was shifted to the right and upward, while at low LC$_{csp}$, the curve was shifted to the left and downward compared with the control curve (100 mm Hg LC$_{csp}$). When the LC$_{csp}$ was set at 60 mm Hg, the baseline pressure was 118±4 mm Hg at the beginning of the conditioning period and 104±3 mm Hg at the end of the conditioning period ($p<0.05$). On the other hand, when the LC$_{csp}$ was set at 140 mm Hg, the baseline pressure was 93±4 mm Hg at the beginning of the conditioning period and 102±5 mm Hg at the end of the conditioning period ($p<0.05$).

### Resetting Indexes and Extent of Resetting

**Resetting indexes.** In six dogs, the four parameters we chose for evaluating resetting were derived for the RCSP-MAP baroreflex curves. The average values for $P_{sh}$, $P_{sp}$, $BP_{50}$, and $GM_{csp}$ at different LC$_{csp}$ are shown in Figure 6. Compared with the control (LC$_{csp}$=100 mm Hg), all of the indexes are significantly increased at LC$_{csp}$ of 140 and significantly decreased at LC$_{csp}$ of 60 mm Hg. Changes in each of these parameters resulting from the change in LC$_{csp}$ are listed in Table 1. All these changes are statistically significant.

**Extent of resetting.** The extent of resetting was derived from each of the four parameters. The

![Figure 5. A plot of three baroreflex curves constructed from the data shown in Figure 4. The right carotid sinus–mean arterial pressure curves were shifted when the left carotid sinus conditioning pressure (LC$_{csp}$) was set at different levels.](image-url)
Figure 6. The mean data from six dogs are shown in this figure. Four right baroreflex curve parameters that denote resetting are shown. These are the threshold pressure, the "set point" pressure, the midpoint pressure (BP50), which is designated A3 in the equation of Kent et al., and the carotid sinus pressure at which maximum gain occurs (GMcsp). All four parameters were significantly shifted when the left carotid sinus pressure was held at conditioning pressures of 60, 100, and 140 mm Hg. *Significantly different from the 100 mm Hg group.

Changes in each of the four parameters are expressed as a percent of the change of LCapo in both upward and downward directions. However, of these four parameters, the only significant difference between upward and downward resetting was in the Pth. The average extent of resetting derived from the four parameters was 0.55±0.08 mm Hg/mm Hg ΔLCapo when the conditioning pressure was changed downward and 0.37±0.08 mm Hg/mm Hg ΔLCapo when the conditioning pressure was changed by the same amount upward. However, this difference is not significant (p<0.07).

Maximum Responses, Response Range, and Maximum Gain of Right Carotid Baroreflex

The reflex parameters that relate to the buffering function of the baroreflex were derived from each of the three baroreflex curves. These are maximum pressure (Pmax), Pmin, Prange, and Gmax. The response range (Prange) is the difference between the Pmax and Pmin. At high LCapo, both Pmax and Pmin of the right carotid baroreflex were significantly increased compared with the respective values at control LCapo. They were increased by 15±3 and 14±3 mm Hg, respectively. As a result, the Prange at high LCapo is similar to the Prange at control LCapo. These values were 65±9 and 67±7 mm Hg, respectively. On the other hand, at low LCapo, Pmax was significantly decreased by 15±5 mm Hg, but Pmin was decreased by only 7±2 mm Hg. This value is not significantly different from control LCapo. As a result of the nonparallel change in Pmax and Pmin, the Prange at low LCapo was significantly decreased compared with control LCapo. The Prange at low LCapo was 55±6 mm Hg. It was decreased by 12±5 mm Hg from control LCapo. Gmax of the right carotid baroreflex at the low, control, and high LCapos were 1.02±0.17, 1.61±0.32, and 1.61±0.34 mm Hg/mm Hg, respectively. These values are not significantly different from each other.

Discussion

The most important finding in this experiment is the demonstration of a central mechanism in acute baroreflex resetting that is initiated by the baroreceptors. This mechanism is different than that which has been demonstrated through an interaction within the CNS with other efferent inputs. This concept strongly suggests that a change in the input signal from the baroreceptors can change the central set point of the baroreflex.

The evidence for the involvement of a central component in acute resetting was clearly demonstrated in the present experiments. We assumed that both right and left carotid sinuses impinge on a common set of central neurons and effectors. In addition, we assumed that as an integrated reflex, the whole reflex arc, that is, the left carotid baroreceptors, the CNS, and the peripheral vessels, could be influenced by the conditioning pressure. In this experiment, during the conditioning period, the baroreflex was under the influence of the prevailing pressure via the left carotid baroreceptors alone, whereas the right carotid baroreceptors were kept at a constant subthreshold pressure. After the baroreflex was conditioned with a given pressure for 20 minutes, the conditioned left carotid baroreceptors were then set at a subthreshold level, and the right carotid sinus was used to construct baroreflex curves. Therefore, changes in the right carotid baroreflex could not be attributed to the changes in the baroreceptors themselves. Previous evidence has demonstrated that the peripheral vascular tone is not involved in the resetting process per se. Therefore, the changes in the baroreflex observed in this experiment could only be attributed to changes that occurred in the CNS.

It has been suggested that the CNS can be reset by a "central command" mechanism such as occurs...
in exercise\textsuperscript{14} or by the interaction with other reflexes.\textsuperscript{11} However, this mechanism does not appear to play a role in the present experiments. First, the aortic nerves and carotid chemoreceptors\textsuperscript{8,14} were denervated in these dogs. Secondly, although there was a reciprocal change in the baseline arterial pressure at high or low \( \text{LC}_{\text{cs}} \), which may activate the cardiopulmonary receptors and in turn influence the carotid baroreflex, this change was less than 15 mm Hg and is probably not large enough to activate the cardiopulmonary afferents and exert a significant influence on the carotid baroreflex. In addition, studies in anesthetized dogs have shown that the baroreflex resetting process is not significantly influenced by vagotomy.\textsuperscript{15} Finally, although the \( \text{LC}_{\text{cs}} \) was dropped to a subthreshold level, while the right carotid baroreflex was induced, the right carotid sinus may be influenced by the effects of efferent sympathetic fibers that may have been activated by the changes in left carotid conditioning pressure. However, it has been reported that the sympathetic efferent mechanism does not play a significant role in the baroreflex control of blood pressure in anesthetized dogs.\textsuperscript{13} In addition, the \( \text{LC}_{\text{cs}} \) was always reduced to a subthreshold level during construction of the right baroreflex curve; therefore, this condition was the same for all curves. Furthermore, there have been no reports to indicate that these efferent fibers have any sustained effects on the carotid sinus or on the baroreceptors. Therefore, it is unlikely that sympathetic efferent control of baroreceptor discharge can account for these experimental results. The above discussion indicates that the direction of baroreflex resetting depends on the input from the left carotid sinus nerve under the conditions of the present experiments. Figure 7 illustrates the possible neuronal mechanism(s) for baroreflex resetting. Baroreceptors send information concerning the change in blood pressure to the CNS. After initial processing and integration at the primary neuronal station, the error is then determined by comparing the integrated input signal with the set point. Depending on the error, a decision is made to activate the efferent neurons, that is, the controller. Therefore, the appropriate negative feedback adjustment of the cardiovascular system is carried out at the effector organs. The set point of the baroreflex is determined by a group of neurons in the CNS. These neurons could be influenced in two different ways, one being by the activity of adjacent neurons that may be activated by other afferents or from another part of the CNS. Secondly, the "set point" could be influenced by the input signal from the baroreflex arc itself. As shown in Figure 7 by the dashed line, it is likely that there is a pathway that allows the baroreceptor input signal to directly influence the neurons that determine the reflex set point. If this is true, this indicates that the CNS can respond to changes in the baseline level or discharge pattern of the input signal and readjust its set point. Since the nucleus tractus solitarii is the primary center where there are mutual projections from the right and left carotid sinuses,\textsuperscript{16} the above central resetting mechanism may occur at or above the nucleus tractus solitarii level.

It is important to understand the degree of acute resetting that can be accomplished by this central mechanism and the relation between receptor resetting and central resetting. In the present experiments, at high \( \text{LC}_{\text{cs}} \), the CNS alone can account for resetting to the degree of 40\%\textsuperscript{+}8\% of the conditioning pressure. Reports in conscious rabbits indicate that the \( \text{P}_{\text{a}} \) of aortic nerve activity can reset 46\% \( \Delta/\text{mm Hg of MAP with sustained hypertension}.\textsuperscript{17} \) In a previous study done in conscious dogs, we demonstrated that the \( \text{P}_{\text{a}} \) of the baroreflex can reset by 59\%\textsuperscript{+}11\% of the conditioning pressure during 20 minutes of high carotid sinus pressure.\textsuperscript{4} It is obvious that along the baroreflex arc, the receptors and the central components did not show simple summation during acute resetting; rather, there is substantial redundancy in this system. The structural
and functional changes in the receptors themselves may not be necessary for inducing acute baroreflex resetting, since acute resetting can occur by a purely central mechanism. The concept that the CNS can adjust its responsiveness to the afferent input is quite different than the traditional concept. In the traditional concept, the central component only passively responds to the afferent input; therefore, the change in the responsiveness of the receptors has been considered as the primary contributor to acute baroreflex resetting. 7 The CNS may play a more comprehensive role in integrating, recognizing, and processing the afferent information than that which has been appreciated. However, the accurate location of those neurons that are responsible for the set point of the baroreflex and the mechanism of their modulation is beyond the scope of the present experiments and remains to be determined.

Another important aspect of this study is the interaction between left and right carotid baroreceptors. Previous studies have been able to show some interaction between carotid sinus baroreceptors in anesthetized animals. 3,13,16,18,19 However, ours is the first study to show an interaction between the carotid sinuses in conscious animals. First, in the present study, conditioning one carotid sinus could significantly influence the contralateral reflex set point but not the maximum gain. Secondly, in our previous study 4 in which both carotid sinuses were conditioned, the operating range at the control conditioning pressure was 81±5 mm Hg. In the present study, the operating range of right carotid baroreflex alone was 67±7 mm Hg at the same conditioning pressure (100 mm Hg). Therefore, the operating range of one side can compensate about 82% of the range observed when both carotid sinuses are conditioned. This is different from the results of studies in anesthetized dogs. 18,19 Finally, the maximum depressor response of the right baroreflex was similar to that when both carotid sinuses were stimulated. 4 This supports the studies in anesthetized animals that showed that one carotid sinus can fully compensate for the depressor effect of both carotid sinuses. 20

In summary, the present study demonstrates that a short-term change in the conditioning pressure of the baroreceptors can reset the central set point of the baroreflex. The extent of central resetting is similar to the extent of baroreceptor resetting. However, reflex resetting by this central mechanism did not show summation with baroreceptor resetting. It is possible that in the conscious state, the baroreflex can rapidly reset its set point within the CNS in response to hypertension or hypotension. This study also demonstrates that ipsilateral carotid sinus hypertension or hypotension can selectively reset the contralateral carotid baroreflex set point but not the maximum gain.

References

Key Words • baroreflex • resetting • central nervous system • hypertension • carotid sinus • conscious dogs
A central mechanism of acute baroreflex resetting in the conscious dog.
W Tan, M J Panzenbeck, M A Hajdu and I H Zucker

Circ Res. 1989;65:63-70
doi: 10.1161/01.RES.65.1.63
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4371

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/65/1/63

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/