Autoregulation of the Systemic Circulation in Conscious Rats

CARMEN HINOJOSA-LABORDE, ANDREW S. GREENE, AND ALLEN W. COWLEY, JR.

SUMMARY  Autoregulation of blood flow in various organ systems is a well-documented phenomenon. However, the net effect of these regional autoregulatory responses on the systemic circulation has not been studied in conscious rats despite the now extensive use of rats in cardiovascular research. The ability of the systemic circulation to autoregulate cardiac output has been proposed to play an important role in the development of increased vascular resistance in volume-dependent forms of hypertension. To better understand these events, we characterized responses to acute increases and decreases in blood volume in conscious areflexic rats that were chronically instrumented with arterial and venous catheters and an electromagnetic flow probe around the ascending aorta. Neurohumoral blockade was achieved with chlorisondamine (10 mg/kg), methscopolamine (0.5 mg/kg), captopril (1.0 mg/kg), and d(CH2)5Tyr(Me)arginine vasopressin (10 ng/kg). Mean arterial pressure was restored to normal levels with a constant i.v. norepinephrine infusion, which resulted in normal values of cardiac output, total peripheral resistance, and blood gases. Blood volume expansion (0.9 ml i.v. blood infusion for 6 minutes) increased cardiac output 9 ± 1% and mean arterial pressure 30 ± 3% and caused a 22 ± 2% increase in total peripheral resistance (n = 7). Blood volume contraction (6-minute withdrawal of 0.9 ml of blood) decreased cardiac output 12 ± 1% and mean arterial pressure 26 ± 4%, which resulted in a 16 ± 4% decrease in total peripheral resistance (n = 8). The slopes of the pressure-flow relationships during volume expansion were 0.24 and 0.41 during volume contraction, as compared with a nonautoregulating system (slope = 1) and a completely autoregulating system (slope = 0). We conclude that conscious rats exhibit a high degree of whole-body autoregulation in response to only 5% increases or decreases in blood volume. (Hypertension 11: 685–691, 1988)
physiological conditions. Furthermore, whole-body autoregulation has not been previously quantified in normal, conscious rats, and since the rat is the animal most commonly used in experimental models of hypertension, it is important to quantify these responses in this species. The present study was therefore designed to characterize whole-body autoregulation in response to acute expansion and contraction of blood volume in conscious rats chronically instrumented with arterial electromagnetic flow probes for cardiac output measurements.

Materials and Methods

Animal Preparation
Male Sprague-Dawley rats (King Animal Labs, Madison, WI, USA) weighing 300 to 350 g were anesthetized with sodium pentobarbital, 65 mg/kg, administered intraperitoneally. At this time the animals were also given an intraperitoneal injection of atropine sulfate (0.4 mg/kg) to depress respiratory tract secretions. The rats were intubated and respired with room air using a rodent respirator (Model 680, Harvard, South Natick, MA, USA). A left-sided thoracotomy through the third intercostal space was performed to expose the ascending aorta. This portion of the aorta was cleared of surrounding connective tissue to allow for the proper fit of an electromagnetic flow probe (Series EP100, Carolina Medical Electronics, King, NC, USA). After placement of the flow probe, the chest cavity was closed and air in the chest cavity was removed by suction to reestablish a negative intrathoracic pressure. The flow probe cable was secured subcutaneously around the left thoracic area, and the connector end of the flow probe was exteriorized dorsally. Rats were treated postoperatively with penicillin G (20,000 units) and allowed to recover for 3 to 5 days.

In a second surgical procedure, the rats were anesthetized with methoxyflurane and instrumented with an arterial catheter for the measurement of arterial pressure and with venous catheters for infusions. Catheters were placed in the left femoral artery, left femoral vein, right femoral vein, and right jugular vein. The catheters were tunneled subcutaneously and exteriorized at the back of the neck. The animals were allowed to recover for 1 to 2 days before the experimental protocol.

Experimental Protocol
On the day of experimentation, the rat was placed in a Plexiglas restrainer inside a small, soundproof chamber. A mixture of 21% oxygen and 79% nitrogen was circulated continuously through the chamber at a rate of 2 L/min. The oxygen content of the gas mixture was monitored with a polarographic oxygen sensor (Model IL406, Instrumentation Laboratory, Lexington, MA, USA) attached to the inlet hose. The arterial and venous catheters were connected to extension tubing, and the electromagnetic flow probe was connected to a lightweight extension cable that exited the chamber through a small, sponge-padded opening. The arterial catheter was attached to a pressure transducer (Model P23Dd, Statham, Oxnard, CA, USA) to measure arterial pressure, and cardiac output was measured by an electromagnetic flowmeter (Model 501D, Carolina Medical Electronics). Measurements of mean arterial pressure (MAP) and cardiac output (CO) were recorded on a Grass polygraph recorder (Model 7D, Quincy, MA, USA). Total peripheral resistance (TPR) was calculated as the ratio of MAP to CO.

Resting MAP and CO were recorded for approximately 1 hour while the rat became accustomed to its surroundings. Following this stabilization period, neurohumoral reflex function was abolished with pharmacological blockade. Ganglionic transmission of the autonomic nervous system was blocked with an i.v. injection of chlorisondamine chloride, 10 mg/kg, and methscopolamine bromide, 0.5 mg/kg. Angiotensin II synthesis was blocked by i.v. administration of captopril, 1 mg/kg, an angiotensin converting enzyme inhibitor. The vasoconstrictor effect of vasopressin was abolished by an i.v. injection of d(CH2)5Tyr(Me)arginine vasopressin, 10 μg/kg, a specific vascular vasopressin receptor antagonist. In addition to the initial bolus injections of the blocking agents, a cocktail containing these drugs was continuously infused intravenously by infusion pump (Model 355, Sage Instruments, Boston, MA, USA) at a very slow rate of 0.015 ml/min. The concentrations of the blocking agents in the solution were calculated to deliver the initial bolus dose of each of the agents over a 1-hour period. MAP and CO in reflex-ablated animals were maintained at normal levels with a constant i.v. infusion of norepinephrine (0.5–1.0 μg/kg/min). Although the neurohumoral blocking agents and norepinephrine were administered intravenously in saline, a special effort was made to minimize the volume of saline introduced into the circulation. We calculate that, during an hour of infusion of blocking drugs and norepinephrine, the animal received approximately 1.2 ml of saline. Assuming the rats had normal renal function, these amounts of saline will not affect the volume status of the rat.

The doses of blocking agents used in this study were previously tested for completeness of blockade in another group of rats. In preliminary studies, before ganglionic blockade with chlorisondamine chloride (10 mg/kg) and methscopolamine bromide (0.5 mg/kg), angiotensin I (200 ng/kg) increased MAP 38 ± 3 mm Hg and decreased heart rate 51 ± 13 beats/min. After ganglionic blockade, an equipressor dose of angiotensin I (25 ng/kg) caused MAP to increase 37 ± 7 mm Hg while heart rate did not change (−1 ± 2 beats/min). Captopril (1 mg/kg) abolished the pressor effect of angiotensin I (50 ng/kg) by 93 ± 3%, and d(CH2)5Tyr(Me)arginine vasopressin (10 μg/kg) caused a 98 ± 2% reduction of the pressor effect (32 ± 4 mm Hg) of vasopressin, 15 mU/kg. Following neurohumoral reflex ablation, the rats were divided into two groups. Group 1 rats (n = 7) were subjected to an acute expansion of blood volume. In these rats, donor blood (see the next section for preparation) was infused through a venous catheter at a
rate of 0.15 ml/min using an infusion pump (Sage Instruments). A total volume of 0.9 ml of donor blood was infused in 6 minutes. Approximately 3 to 5 minutes after the infusion of donor blood, the excess blood volume (0.9 ml) was withdrawn through the arterial catheter to restore blood volume to control levels. This blood was used to determine blood pH, oxygen tension, and carbon dioxide tension using an ABL-2 blood gas analyzer (Radiometer, Copenhagen, Denmark).

Rats in Group 2 (n = 8) were subjected to an acute decrease in blood volume. Blood was withdrawn from the right atrium through the jugular vein catheter at a rate of 0.15 ml/min for 6 minutes. The total volume of removed blood was 0.9 ml. Approximately 3 to 5 minutes later, the blood was reinfused into the jugular catheter to restore blood volume to control levels. In addition, 2 minutes after replacement of the withdrawn blood, a 0.25-ml arterial blood sample was taken to determine acid-base levels and blood gas content.

**Preparation of Donor Blood**

Blood volume expansion was achieved by an infusion of donor blood. Since whole blood could not be used in our preparation because of the presence of vasoactive substances in the plasma, the following procedure was followed to prepare a suspension of red blood cells and thus eliminate the plasma and platelet components of whole blood. On the morning of an experimental protocol, a Sprague-Dawley rat was exsanguinated to obtain 12 to 15 ml of donor blood. The blood was collected in a large syringe containing approximately 5 ml of an anticoagulant solution (Aloeever’s solution), and the mixture was centrifuged immediately for 20 minutes at 600 rpm in a Dynac centrifuge (Becton-Dickinson, Parsippany, NJ, USA). After the plasma was removed and discarded, the red blood cells were resuspended in physiological saline solution with a pH of 7.4. After gentle mixing, the suspension was centrifuged for 20 minutes at 600 rpm. The supernatant solution was discarded, and the red blood cells were washed again with the physiological saline solution. After two wash sequences, the red blood cells were resuspended in a protein solution (physiological saline solution + 5% albumin + 2% globulin) to mimic the oncotic properties of plasma and titrated with 1 N sodium bicarbonate to correct for acid-base imbalances. Before use of the donor blood in an experimental protocol, blood electrolytes were analyzed using a Nova-1 analyzer (Nova Biomedical, Boston, MA, USA) and blood gases and blood pH were evaluated using an ABL-2 blood gas analyzer (Radiometer) to verify that these parameters were within a normal physiological range.

**Statistical Analysis**

The results of this study are expressed as means ± SEM. One-way analysis of variance with repeated measures was used to evaluate the changes in MAP, CO, and TPR in response to acute increases and decreases in blood volume. The Dunnett’s multiple range test was used to determine significant differences in these hemodynamic changes when compared with control values. Differences between baseline hemodynamic variables before and after pharmacological blockade of neurohumoral reflex systems were assessed with a Student’s t test for paired values. Differences in hemodynamic values, blood gases, and blood pH between Groups 1 and 2 were determined by a Student’s t test for unpaired values. Linear regression analysis was used to determine the slopes of the pressure-flow relationships during increases and decreases in blood volume followed by an unpaired Student’s t test to compare the slopes of these lines. A probability level below 0.05 was considered statistically significant for all tests.

**Results**

To assess the adequacy of the slow infusion of norepinephrine in restoring the hemodynamic variables to control state, MAP, CO, and TPR were compared before and after pharmacological blockade (Table 1). Although there was a greater variability in resting MAP in areflexic rats, there were no significant differences in hemodynamic variables between the intact and areflexic states of Groups 1 and 2. Thus, the slow, continuous infusion of norepinephrine used to restore MAP to resting control levels was effective in reestablishing normal control levels of CO and TPR before the volume-induced changes. In addition, the oxygen tension, carbon dioxide tension, and pH values were 91 ± 7 mm Hg, 39 ± 1 mm Hg, and 7.43 ± 0.01, respectively, in Group 1, and 105 ± 6 mm Hg, 41 ± 1 mm Hg and 7.40 ± 0.01, respectively, in Group 2 during the areflexic state. These values were well within the normal physiological range, and there were no significant differences in these values between Groups 1 and 2.

Figure 1 represents the average hemodynamic values observed before and during the 6-minute infusion of blood in Group 1. The expansion of blood volume resulted in progressive elevations of MAP and CO during the 6-minute volume infusion period. MAP, however, rose proportionately about three times more than CO during the infusion. The technical limitations of CO measurements would not be expected to detect very small increases that may have been present during the first minute. Based on the responses seen in the succeeding 5 minutes of infusion, the data indicate that

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (n = 7)</th>
<th>Group 2 (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>113 ± 3</td>
<td>114 ± 3</td>
</tr>
<tr>
<td>Cardiac output (ml/min)</td>
<td>98 ± 6</td>
<td>107 ± 5</td>
</tr>
<tr>
<td>Total peripheral resistance (mm Hg·min·ml⁻¹)</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SEM.
very small increases in CO had pronounced effects on MAP and TPR, which increased significantly within 1 minute after the start of infusion and approached a plateau during the last 3 minutes of the infusion. Thus, at the end of the infusion, after a blood volume expansion of approximately 5% (0.9 ml), CO had increased by 9 ± 1% and MAP had increased by 30 ± 3%, which resulted in a maximum 22 ± 2% increase in TPR as compared with control values at Time 0.

Figure 2 shows the hemodynamic responses to a removal of blood in Group 2. Blood withdrawal caused a progressive fall in CO. Significant decreases also occurred in MAP and TPR, and these values continued to decrease during the removal of blood. Thus, the removal of approximately 5% of blood volume (0.9 ml) caused a 12 ± 1% decrease in CO and a 26 ± 4% decrease in MAP, which resulted in a 16 ± 4% decrease in TPR.

The systemic pressure-flow relationships during increases and decreases in blood volume for the individual animals are summarized in Figure 3. The data are plotted as the fractional change in pressure (ΔP/P) and the fractional change in flow (ΔF/F) during each minute of blood infusion and blood withdrawal. ΔP represents the difference between the MAP at 1-minute intervals and MAP at Time 0 (P). ΔF represents the difference between CO at the same 1-minute intervals and CO at Time 0 (F). The ratios of ΔP/P and ΔF/F were determined prior to volume infusion or withdrawal (Time 0) and at 1-minute intervals during the 6-minute protocol. Thus, the systemic pressure-flow relationships were determined by seven points in each rat with linear regression analysis. Also shown in Figure 3 are the theoretical pressure-flow relationships predicted for a rigid, nonautoregulating system and for a completely autoregulating system. In the case of a rigid, nonautoregulating system in which there is no change in resistance, a fractional change in pressure is associated with a similar fractional change in flow. A completely autoregulating system would demonstrate no change in flow regardless of changes in pressure because resistance changes would offset pressure changes to maintain flow.

The autoregulatory capacity of the systemic circulation can be represented as the slope of the regression lines shown in Figure 3. During volume expansion, the
slopes of the pressure-flow relationship is 0.24, while during volume depletion the slope is 0.41, as compared with a nonautoregulating system (slope = 1) and a perfectly autoregulating system (slope = 0). Since the slopes were not statistically different, these results indicate that the areflexic rat has a similar autoregulatory capacity during increases in blood volume and decreases in blood volume.

Discussion

The present study indicates that, in the absence of the major neural and humoral reflex mechanisms, the rat is capable of changing vascular resistance to regulate CO during both acute increases and decreases in blood volume. Special measures were taken to avoid the complicating factors of anesthesia and surgery, which have plagued the interpretation of previous studies in this field. It was necessary to remove the neurohumoral controllers of blood flow to selectively assess local regulatory processes. Following pharmacological blockade, normal levels of MAP, CO, and TPR were restored with an infusion of norepinephrine. This allowed us to quantify autoregulation in the presence of normal levels of vascular tone. In addition, the rats were studied in a conscious state while blood gases and blood pH were within the normal range. Despite the interventions required for the evaluation of autoregulation, the rats displayed grooming behavior without signs of distress or discomfort. During the experimental protocol, minimal amounts of saline were infused into the animal to prevent changes in volume. In addition, special procedures were followed in the preparation of donor blood to eliminate vasoconstrictor substances in the plasma and to resuspend washed red blood cells in a plasmalike protein solution.

Since whole-body autoregulation has been hypothesized to contribute to the elevated systemic vascular resistance during volume-dependent forms of hypertension, our purpose in this study was to characterize the autoregulatory hemodynamic responses not only to decreases but also to increases in blood volume. The onset of volume-dependent hypertension is characteristically associated with a 28% elevation in blood volume. In our experiments, an infusion or withdrawal of 0.9 ml of blood represents an approximate 5% change in blood volume. Since we used resuspended red blood cells rather than whole blood, we are confident that the hemodynamic effects of donor blood infusion are a result of volume expansion rather than direct vasoconstrictor effects. Our results indicate that increases in blood volume of only 5% in conscious rats increase TPR by 22%, and this suggests that small, almost undetectable increases in blood volume are capable of causing significant autoregulatory increases in vascular resistance. Thus, our findings provide support for the hypothesis that whole-body autoregulation contributes to the increased systemic resistance in volume-dependent hypertension that is characteristically associated with increases in blood volume greater than 5%.

Because autoregulatory responses provide an important contribution to the local control of hemodynamics in the normal cardiovascular system, many investigators have studied autoregulation responses in the systemic circulation. Several studies demonstrated that autoregulation produced results qualitatively similar to our results; however, these studies were performed in anesthetized, decapitated, or pump-perfused animals. Other investigators have been unable to consistently demonstrate autoregulation in similar preparations. These differences may be due to the widely differing techniques used for stimulation of the autoregulatory response. Ehrlich et al. measured pressure-flow relationships in conscious dogs; however, an evaluation of the autoregulatory response was not possible in this study since these dogs were not neurohumorally blocked.

In our study we expressed the strength of the autoregulatory responses as the slope of the normalized pressure-flow curve. This method of evaluation is based on the theoretical results indicating that the slope of these pressure-flow curves will range between 0 and 1, depending on the degree of change in resistance of the system. The autoregulatory capacity of the systemic circulation has been expressed as the open-loop gain of a control system by Granger and Guyton. Using
this technique, they calculated an open-loop gain of 3.3 for the autoregulatory control system. If we assume that a steady state condition occurred during blood infusion in our experiments, as indicated by the plateau in MAP and TPR after 3 minutes (Figure 1), we calculate an open-loop gain of 2.8 for systemic autoregulation during volume expansion. Thus, both the pressure-flow slope and the "autoregulatory gain" are indicative of an important local control of blood flow in the rat.

The studies that have used local autoregulation to explain changes of TPR with alteration of blood volume have been observations obtained in the absence of a reflex-modulating system. In the present study, additional steps were taken to block not only the autonomic nervous system, but also the renin-angiotensin system and the vasopressin system. Despite blockade of these major, rapidly acting, pressure control systems, other circulating vasoactive substances may have modulated the observed responses.

A hormone that was not controlled in our experiment, and that may affect blood pressure and body fluid composition, is atrial natriuretic factor. The release of this peptide is thought to be linked to atrial distention caused by an increase in cardiac filling volume. In the present study, a 5% increase in blood volume may have stimulated the release of atrial natriuretic factor, causing natriuresis and diuresis and a lowering of blood pressure. Since neither atrial natriuretic factor levels nor sodium and water excretion was measured in our rats, it is difficult to assess the role of this hormone in our experiments. However, atrial natriuretic factor probably is not responsible for the hemodynamic effects of blood volume expansion, because it is unlikely that the natriuretic and diuretic effects are expressed during a 6-minute infusion of blood. In addition, even if atrial natriuretic factor were affecting electrolyte excretion, the hemodynamic effects would decrease TPR rather than increase TPR, as was observed with volume expansion.

Certain other aspects of the present study should be recognized. First, the changes in resistance observed could be explained by a putative ouabainlike factor that is believed to be produced in the central nervous system and released during volume expansion. This factor is hypothesized to cause inhibition of Na\(^+\),K\(^+\)-adenosine triphosphatase pump activity and an increase in vascular reactivity. Since the levels of the putative ouabainlike factor are hypothesized to be elevated during volume expansion, they could account for the observed increases in TPR. Similarly, decreased levels of this factor during volume contraction could account for the decrease in TPR. However, observations in a small number of decapitated rats in our laboratory (n = 3; unpublished observations, 1987) provide evidence that the centrally released ouabainlike factor is not mediating the changes in TPR. Autoregulation responses similar to those seen in conscious rats were observed during volume expansion in these animals.

Second, not all rats exhibited autoregulatory responses during increases or decreases in blood volume. TPR did not change in approximately 25% of the rats we observed (not included in the results of this study) in response to a 5% change in blood volume. The hemodynamic responses of these rats were similar to what would be expected of a passive circulatory system in which changes in CO cause similar changes in MAP without affecting TPR. Nonautoregulating rats were instrumented and subjected to the same procedures as autoregulating rats. There were no consistent differences in baseline hemodynamics or blood gas values between these two groups. Therefore, we were unable to explain the lack of autoregulation in these rats.

Finally, we expect that the information obtained from this study will have important implications on the role of whole-body autoregulation in the development of volume-dependent hypertension. We realize that the autoregulatory responses observed in this study represent the immediate response to volume expansion and that the hemodynamic and vascular response to volume expansion may be modified over time. However, we predict that these acute responses are the initial step in the sequence of events leading to the chronic stage of volume expansion. Thus, the evaluation of the acute autoregulatory response to volume expansion is an important step leading to the understanding of volume-dependent hypertension.

In summary, our study indicates that conscious areflexic rats are able to autoregulate CO during acute increases and decreases in blood volume. To our knowledge, these autoregulatory responses have not been demonstrated previously in rats or during a conscious state. Our results show that autoregulatory changes in vascular resistance occur during 5% changes in blood volume and that these autoregulatory responses may play an important role in the regulation of cardiovascular homeostasis in normal states and in disease states such as hypertension.

Acknowledgments
The generous donations of chlorisondamine chloride by CIBA Pharmaceutical Company and captopril by the Squibb Institute for Medical Research are gratefully acknowledged.

The authors thank Rosalie Zamiatkowski for technical assistance and Meredith Skelton for her assistance in data analysis and creation of the figures. Therese Gauthier and Michelle Rossa are acknowledged for their secretarial assistance.

References
5. Folkow B. A study of the factors influencing the tone of dener-
Autoregulation of the systemic circulation in conscious rats.
C Hinojosa-Laborde, A S Greene and A W Cowley, Jr

Hypertension. 1988;11:685-691
doi: 10.1161/01.HYP.11.6.685

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/11/6_Pt_2/685

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Hypertension 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 Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/