Baroreceptor Reflex Influence on Peripheral Circulations in Salt-Loading Hypertension in Dogs

JEAN-FRANCOIS LIARD, M.D., AND ROSELLA SILENZIO, M.D.

SUMMARY To determine the importance of cardiovascular reflexes in the vasodilation found in skeletal muscle in the early stages of salt-loading hypertension in dogs, we gave for several days an intravenous infusion of isotonic sodium chloride, 190 ml/kg/day, to seven dogs with their renal mass reduced following extensive destruction of their arterial baroreceptor afferents. Mean arterial pressure, cardiac output (electromagnetic flowmeter), and regional blood flows (radioactive microspheres) were measured sequentially and the results compared with those obtained in five control dogs. The salt-loaded animals exhibited an increase in cardiac output and in arterial pressure on the first day of infusion. Blood flow to the splanchnic area, the skin, the bone, the skeletal muscle, the heart, the lungs and the brain increased significantly, but vascular conductance was unchanged in most territories, including skeletal muscle. After 5 to 7 days, cardiac output returned toward control values, but pressure remained elevated. Apart from the myocardium, the regional blood flows were normalized and the conductances were decreased. These results indicate that cardiovascular reflexes are responsible for the transient early vasodilation in skeletal muscle which characterizes salt-loading hypertension in intact animals. (Hypertension 4: 597-603, 1982)

KEY WORDS • blood volume • cardiac output • hemodynamics • microspheres • regional blood flows • sodium • water-electrolyte balance • vascular resistance

We recently reported that hypertension resulting from salt and water loading after renal mass reduction in dogs was characterized by inhomogeneity of vascular resistance changes in various individual beds during the initial phase of high cardiac output. Thus, the increase in systemic blood flow was almost entirely diverted to the skeletal muscle vascular bed that exhibited significant vasodilation, whereas most of the other territories showed some degree of vasoconstriction very early with no evidence of increased perfusion. The changes in regional vascular resistance observed made it obvious that consideration of total peripheral resistance alone could not provide a meaningful account of the vasoconstrictor processes which take place in this type of hypertension, especially with respect to their progression with time.

The reason for the early vasodilation in skeletal muscle found in our previous study was not clear. It might have been mediated by the nervous control of the circulation, since both high- and low-pressure mechanoreceptors were stimulated during the initial stage of salt and water loading by the rise of blood pressure and blood volume. However, it is not clear why this reflex control would have the skeletal muscle as the only target. Whatever the explanation proves to be, the capacity of the muscular vascular bed to increase its flow appears to be of significance in several situations since it has also been reported in acute extracellular volume expansion in anesthetized rats and in three models of rat hypertension. However, it does not seem to be present in man during acute volume expansion nor in rats during chronic dietary saline loading.

In spontaneously hypertensive rats, an increased resistance of the microvascular network has been described at an early age in the spinotrapezius muscle.

In the present study we repeated our previous experiments in dogs surgically deprived of a number of cardiovascular afferents, in particular from the arterial baroreceptors. This procedure has been shown to accelerate the development of salt-loading hypertension without affecting the final steady state level.
Methods

Animal Preparation

Twelve male mongrel dogs were used for these experiments from a total of 21 animals initially included in the study. Body weight on Day 0 of the protocol described below was 17.2 ± 0.9 kg. The dogs underwent four surgical interventions under pentobarbital anesthesia (30 mg/kg, supplemented as necessary) and in sterile conditions. First, the two poles (9.3 ± 0.7 g) of the left kidney were excised. Four weeks later, the right kidney was removed (44.2 ± 2.2 g). By comparison, the weight of the left kidney at the end of the experiment was 57.4 ± 3.6 g. After another 4 weeks the animals were equipped with arterial and venous catheters placed in the abdominal aorta and inferior vena cava through the right external iliac vessels. They were also subjected to a denervation procedure, which included surgical stripping of both carotid sinuses, painting of the area with phenol, and total resection of the left as well as partial resection of the right vagosympathetic trunk. This procedure destroyed a major portion of the arterial baroreceptor and chemoreceptor afferents, as indicated by usual test procedures, but also a number of afferents from low-pressure mechanoreceptors and of vagal efferents as well. Several dogs were rejected from the study following this intervention because of insufficient barodenervation or of intractable vomiting.

Two weeks after the denervation, the dogs were subjected to the last surgery with a reduced dose of pentobarbital. Through a thoracotomy at the fourth intercostal space, an electromagnetic flow transducer was placed round the root of the aorta for cardiac output measurement (without coronary blood flow) and a catheter was implanted into the left atrium for microsphere injections. All cables and catheters were exteriorized on the back of the animals and protected by a jacket.

Measurement of Systemic Hemodynamics and Regional Blood Flows

All measurements were performed in the awake animals using techniques identical with those described previously. Briefly, arterial pressure (mean and pulsatile), cardiac output (computed from the beat-to-beat integration of the aortic flow signal), heart rate, and left atrial pressure were recorded on a multichannel paper oscillograph. The analysis of the recorded data was performed in the following manner: a value for each variable was read from the paper record every 20 seconds and used for subsequent calculation of a mean value and standard deviation. This procedure allowed us to define in each dog the average hemodynamic values around which the large fluctuations characteristic of baroreceptor denervated animals occurred. The microsphere injections were done only when conditions as close as possible to this average state prevailed.

Regional blood flows were determined by injecting into the left atrial catheter 15 μm NEN-TRAC microspheres labeled with either 46 Sc, 113 Sn, or 153 Gd, which allowed three measurements in each dog. A reference arterial sample was collected in order to calculate flows to the various organs and tissues following sacrifice of the animals. Details about techniques of injection, tissue sampling, counting, and validation studies can be found elsewhere. The results of regional blood flow measurements were accepted only if hemodynamic values at the time of microsphere injections reflected those characterizing the animal on that particular day. Also, in each dog, the sum of all regional blood flows obtained with one isotope was divided by the cardiac output measured with the electromagnetic flowmeter during microsphere injection. If the ratios obtained at the second and third injections differed from that calculated at the first injection by more than 15%, the experiment was rejected.

Other Measurements

Blood samples were taken from the aortic catheter. Plasma as well as urine sodium and potassium concentrations were measured by flame photometry and hematocrit by microcentrifugation. Plasma volume was measured with Evan's blue dye by extrapolation to zero time from three samples collected 20, 30, and 40 minutes after injection. Blood volume was calculated from plasma volume and uncorrected hematocrit. Urine was collected in a metabolic cage and the daily excretion of sodium and potassium was calculated.

Protocol for Salt Loading

Following recovery from the last surgery, the dogs were trained to lie quietly in the recording pen for several hours. Then, a 5-day control period was started during which hemodynamic measurements were collected as well as two measurements of blood volume and plasma electrolytes. On the last day of the control period (Day 0), the first injection of microspheres was performed to obtain control regional blood flows. Then a continuous infusion of sterile sodium chloride solution, 156.2 ± 1.0 mM, was started in seven dogs at a rate of 189.6 ± 5.4 ml/kg/day. On Day 1, systemic hemodynamics, regional blood flows, blood volume, and plasma electrolytes were measured again. All measurements were repeated 5 to 7 days after the start of the infusion. In five dogs serving as controls, no isotonic saline infusion was given, but the same repeated measurements were performed. Food intake was maintained constant throughout the experiment, by force-feeding when necessary.

Statistical Analysis

Results are given as means ± SE. Changes from control values were calculated on Day 1 and on Days 5-7 by subtracting the average values measured during the control period from the values measured on these particular days. The significance of these changes was assessed by a paired t test. Linear regressions and correlations were calculated with the least-squares method. Statistical significance was accepted for a p value less than 0.05.
Results

Humoral Changes and Balance Studies

Salt- and Water-Loaded Dogs

Body weight increased by 1.0 ± 0.2 kg on the first day of infusion. It did not differ from its control value on the last day. Blood volume was 1494 ± 127 ml (86.4 ± 2.3 ml/kg) in the control period and increased by only 64 ± 41 ml (not significant) on Day 1. It remained close to its control value on the last day of the infusion. Hematocrit was 34.7 ± 1.6% during the control period and plasma sodium was 146.4 ± 0.9 mEq/liter; it increased on Day 1 by 1.6 ± 0.5 mEq/liter and remained elevated. Plasma potassium did not change from its control value of 3.59 ± 0.09 mEq/liter.

Urinary volume was 620.6 ± 89.7 ml/day in the control period. It increased by 1542 ± 337 ml on Day 1; by 2286 ± 335 ml on Day 2; and by 2978 ± 402 ml on Day 3, after which time it remained stable. Water intake was 512.3 ± 121.8 ml/day in the control period and was markedly reduced to 198.1 ± 92.3 ml/day during salt- and water-loading. Control urinary excretion of sodium was 85.4 ± 11.8 mEq/day. It increased by 270.9 ± 44.8 mEq on Day 1; by 462.5 ± 34.6 mEq on Day 2; and by 609 ± 112.2 mEq on Day 3, after which time it remained stable and approximately equal to the amount of sodium infused plus the amount contained in the food.

Control Dogs

Body weight did not change significantly, nor did blood volume, plasma sodium, and potassium concentration. Urinary volume and sodium excretion remained constant throughout the experiment.

Systemic Hemodynamics

For the control values at the time of the first microsphere injection, results from the seven dogs that were to receive the saline infusion and the five control dogs have been combined. Mean arterial pressure was 112.3 ± 6.2 mm Hg (n = 12); cardiac output 2264 ± 147 ml/min; and the ratio of mean arterial pressure and cardiac output, defined as total peripheral resistance, was 50.7 ± 3.4 mm Hg min/liter. Heart rate was 126.2 ± 6.3 beats/min, and left atrial pressure was −1.0 ± 1.2 mm Hg. These values obtained at one point in time reflected well those calculated from long-term recording with repeated sampling. The only significant difference between the two sets of values by paired t test was for cardiac output, which was about 10% lower at the time of microsphere injection, presumably reflecting the fact that the dogs were particularly quiet. As usual, the arterial pressure of baroreceptor-denervated dogs was characterized by very pronounced variability, as shown by the large average standard deviation calculated from long-term recordings (19.5 ± 1.6 mm Hg for periods of several hours, with about 300 data points).

Figure 1 represents the changes in mean arterial pressure, cardiac output, and total peripheral resistance measured at the time of the second and the third microsphere injection in both the salt- and water-loaded and the control groups. In salt-loaded animals, pressure increased on the first day and remained elevated. Cardiac output rose by 47% initially, then went back toward control. Total peripheral resistance was increased only secondarily. These results closely reflected those that were calculated from the repeated sampling of continuously recorded hemodynamic data. Heart rate increased by 14.9 ± 6.6 beats/min on the first day of salt loading, and left atrial pressure increased by 5.4 ± 0.6 mm Hg; it then remained elevated for the duration of the infusion. Neither variable changed significantly in the control group.

Regional Blood Flows

Table 1 summarizes the control values obtained with the first injection of microspheres for organs and tissues which represent a sizable portion of cardiac out-
put. The splanchnic blood flow is the sum of liver, gastrointestinal, spleen, pancreas, and abdominal fat blood flows. For the areas in which blood flow was not homogeneous, no flow per unit weight is provided. Dividing the sum of all but myocardial blood flows by the cardiac output measured with the electromagnetic flowmeter gave a ratio of 1.22 ± 0.07 (n = 12). Vascular conductance was calculated by dividing absolute blood flow by mean arterial pressure, neglecting venous pressure as had been done for total peripheral resistance.

Salt loading induced changes in regional blood flows which are summarized in table 2 for the eight areas previously characterized in intact animals. On Day 1 of salt and water loading, all regional blood flows were increased significantly with the exception of the renal blood flow. This was in striking contrast with what we had observed in intact dogs, as was the

<table>
<thead>
<tr>
<th>Organ</th>
<th>Flow (ml/min)</th>
<th>Conductance (ml min⁻¹ mm Hg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle</td>
<td>703.2 ± 81.9</td>
<td>25.61 ± 0.33</td>
</tr>
<tr>
<td>Heart</td>
<td>163.2 ± 11.4</td>
<td>5.5 ± 0.33</td>
</tr>
<tr>
<td>Kidney</td>
<td>245.9 ± 24.6</td>
<td>8.27 ± 0.66</td>
</tr>
<tr>
<td>Liver</td>
<td>201.9 ± 35.2</td>
<td>6.63 ± 1.12</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>332.6 ± 26.1</td>
<td>11.16 ± 0.7</td>
</tr>
<tr>
<td>Esophagus</td>
<td>15.1 ± 1.3</td>
<td>3.1 ± 0.34</td>
</tr>
<tr>
<td>Stomach</td>
<td>71.0 ± 5.0</td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>56.6 ± 7.4</td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>55.8 ± 4.1</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>81.4 ± 10.4</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>125.1 ± 21.6</td>
<td>4.11 ± 0.64</td>
</tr>
<tr>
<td>Pancreas</td>
<td>69.6 ± 11.4</td>
<td>2.26 ± 0.28</td>
</tr>
<tr>
<td>Abdominal fat</td>
<td>41.9 ± 3.9</td>
<td>1.47 ± 0.17</td>
</tr>
<tr>
<td>Splanchnic</td>
<td>775.8 ± 60.4</td>
<td>25.87 ± 1.28</td>
</tr>
<tr>
<td>Lungs*</td>
<td>161.7 ± 17.5</td>
<td>5.38 ± 0.57</td>
</tr>
<tr>
<td>Brain</td>
<td>76.3 ± 4.5</td>
<td>2.72 ± 0.32</td>
</tr>
<tr>
<td>Skin</td>
<td>294.7 ± 54.3</td>
<td>9.46 ± 1.36</td>
</tr>
<tr>
<td>Bone</td>
<td>335.1 ± 29.5</td>
<td>11.13 ± 0.62</td>
</tr>
</tbody>
</table>

*Significantly different from zero. Values are means ± se.

<p>| TABLE 2. Changes in Regional Blood Flows and Conductances in Seven Salt-Loaded Dogs |
|-----------------------------------|-----------------------------------|-----------------------------------|</p>
<table>
<thead>
<tr>
<th>Day 1</th>
<th>Days 5-7</th>
<th>Day 1</th>
<th>Days 5-7</th>
<th>Day 1</th>
<th>Days 5-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle</td>
<td>+436.9* ± 165.2</td>
<td>−165.5 ± 91.6</td>
<td>+0.77 ± 1.23</td>
<td>−4.16* ± 0.97</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>+150.3* ± 19.3</td>
<td>+87.0* ± 29.1</td>
<td>+0.58* ± 0.13</td>
<td>−0.01 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>+3.5 ± 25.3</td>
<td>+28.3 ± 13.9</td>
<td>−0.63* ± 0.23</td>
<td>−0.44* ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Splanchnic</td>
<td>+250.9* ± 82.6</td>
<td>+111.0 ± 51.9</td>
<td>−0.48 ± 0.63</td>
<td>−1.76* ± 0.69</td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>+50.5* ± 18.6</td>
<td>−3.1 ± 14.1</td>
<td>−0.06 ± 0.15</td>
<td>−0.48* ± 0.11</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>+7.7* ± 3.4</td>
<td>−3.1* ± 0.9</td>
<td>−0.17 ± 0.15</td>
<td>−0.37* ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>+58.4* ± 25.2</td>
<td>+16.0 ± 37.0</td>
<td>−0.42 ± 0.3</td>
<td>−0.71* ± 0.29</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>+105.1* ± 30.9</td>
<td>−14.1 ± 23.6</td>
<td>−0.26 ± 0.25</td>
<td>−1.24* ± 0.18</td>
<td></td>
</tr>
</tbody>
</table>

*Significantly different from zero. Values are means ± se.
absence of a significant correlation between the absolute changes of cardiac output and skeletal muscle blood flow \( r = -0.11 \). After 5 to 7 days of infusion, most blood flows had returned close to their control value. In the control group, none of the changes in blood flow was significant with the exception of a decrease in renal blood flow after 5 to 7 days.

Conductance changes are also reported in table 2. On the first day there was no evidence of vasodilation or constriction in any territory but the myocardium and the kidney. By 5 to 7 days, all areas exhibited a significant decrease in conductance with the exception of the myocardium. This secondary decrease in vascular conductance was especially pronounced in the skeletal muscle.

Figures 2 and 3 illustrate the relative changes in regional blood flows and conductances during the salt and water loading experiment. Figure 4 represents the conductance changes measured in this and in a previous study\(^1\) in still another fashion to emphasize several points. On the first day there was no increase in the portion of systemic conductance accounted for by the skeletal muscle in the baroreceptor-denervated dogs, contrary to what was found in intact dogs. Also, after 5 to 7 days, the portion of the systemic conductance represented by the muscle was decreased, whereas it had simply returned to control in the intact dogs.

Looking in more detail at some of the regional blood flows summarized in table 2, we found that the increase in myocardial blood flow was more pronounced in the left heart. In the splanchnic area, pronounced increases in blood flow took place on the first day in the liver, in the small intestine and in the spleen, but there was no significant increase in the other parts of the gastrointestinal tract nor in the pancreas. In the skeletal muscle, we noted significant increases in flow in the musculus temporalis, masseter, in the muscles of the thoracic wall, the epaxial spinal muscles, the diaphragm, the intercostal muscles, the biceps, the antibrachial muscles, the cranial and caudal muscles of the thigh, and the muscles of the carpus and hindpaw. In the bones, significant increases were observed in the vertebrae, sternum, ribs, scapula, humerus, hip bone, and femur. Among the many regional blood flows that are not included in table 2 because they represent a negligible portion of cardiac output, some showed a significant increase in flow on the first day of salt and water loading (aortic wall, eye), but most did not (salivary glands, testicles, bladder, prostate, thyroid, gallbladder, trachea, pericardium, adrenals).
Another difference between intact and denervated dogs was found in the systemic hemodynamic effects of salt and water loading. The initial increase in cardiac output was more pronounced in the latter. Since this larger change in cardiac output cannot be ascribed to a greater augmentation of the filling pressure of the heart nor to a more pronounced fall in peripheral resistance, it may be due to the absence of a reflex decrease in myocardial contractility following destruction of the baroreceptor feedback loop.

Our results seem to be at variance with those reported by Cowley and Guyton, because at no time during their study was cardiac output higher in baroreceptor-denervated dogs. Cowley and Guyton also reported evidence that total peripheral resistance decreased more in intact than in denervated dogs in the initial stage of hypertension, which differs from the results obtained in our experiments. Resistance changes reflect the influence of several factors, including the vasodilating effects of an increased intravascular pressure and of the cardiovascular reflexes as well as the vasoconstricting effects of autoregulatory processes. It is likely that the relative importance of one or several of these factors differed in both studies. An essential finding in our experiments was that total peripheral resistance increased in intact and baroreceptor-denervated dogs from the first day until the end of the experiment, indicating that baroreceptor adaptation did not explain the progressive secondary increase in vascular resistance.

Regional blood flow measurements brought several pieces of information. First, in the control period, blood flows in intact and barodenervated dogs were very similar. The exceptions were the myocardial blood flow, which was increased following deafferentation presumably because of an elevated heart rate. There was also a substantial increase in cerebral blood flow (22%) and in the flow to the aortic wall in baroreceptor-denervated dogs for which we have no explanation. Cerebral blood flow and mean arterial pressure were not significantly correlated (r = -0.26). Lung blood flow was also increased in barodenervated dogs, but not when normalized per unit weight.

The second information derived concerned the response of the peripheral circulations to salt- and water-loading hypertension. Several differences between intact and baroreceptor-denervated dogs were noted. The denervated dogs did not maintain regional blood flows constant on the first day of salt loading as did the intact animals in most areas. Actually, intact dogs showed an early increase in splanchnic blood flow (22%) and in the flow to the aortic wall in baroreceptor-denervated dogs for which we have no explanation. Cerebral blood flow and mean arterial pressure were not significantly correlated (r = -0.26). Lung blood flow was also increased in barodenervated dogs, but not when normalized per unit weight.

The purpose of the present experiments was to define the importance of the reflex control of the circulation in the pronounced vasodilation that we found in skeletal muscle in the early stage of salt- and water-loading hypertension in dogs. Our results indicate that cardiovascular reflexes played an essential part in this phenomenon.

Dogs studied after baroreceptor denervation exhibited several differences from intact animals although they experienced similar fluid retention as judged from the fluid balance studies and from the change in body weight on the first day of the infusion. Admittedly, we cannot ascribe these differences exclusively to destruction of afferents from the arterial baroreceptors, since other receptors were denervated as well. Among the differences noted was the smaller increase in blood volume after salt loading in deafferented dogs. Since left atrial pressure increased similarly in intact and denervated dogs, this result indicates an impact of the reflexes on the capacitance of the circulation.
Whether the increase in conductance found in this vascular bed in the presence of intact reflexes is due to the selective suppression of sympathetic tone or to activation of a vasodilator pathway cannot be decided on the basis of these experiments. It must be pointed out that the baroreceptor reflex does not seem to operate in saline loading or volume expansion as under normal conditions. However, it seems reasonable to conclude that the nervous control of the circulation is responsible for the inhomogeneous nature of the resistance changes observed in intact animals in the early stages of salt- and water-loading hypertension, and that the operation of the baroreceptor reflex makes it difficult to assess the involvement of other control mechanisms of blood flow through the various tissues.

Common to both groups of dogs was the finding that blood flows were close to normal in all tissues but the heart after 5 to 7 days, a time at which baroreceptors should have adapted in intact animals. Although normal blood flows have not always been found in established hypertension, their existence in a condition characterized by increased vascular resistance suggest that local factors are important in the genesis of the vasoconstriction as pointed out by Coleman et al. Indeed, if humoral or nervous vasoconstriction were responsible, local blood flow abnormalities would likely be present with great frequency. This was the case for instance in a study by Gavras et al., in which increased angiotensin levels were present and several abnormalities in blood flows were observed. Thus our findings indirectly support the concept of autoregulation as defined by others, with the qualification that the local mechanisms controlling blood flow cannot be easily identified when the baroreceptor reflexes are active. The transient overperfusion found in most tissues may well be a factor in the development of increased resistance in the baroreceptor-denervated dogs. These findings relate to the specific model of hypertension described and not necessarily to other forms of hypertension.

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