Circulatory Reflex from Vagal Afferents in Response to Hemorrhage in the Dog

By Conrad L. Pelletier, Anthony J. Edis, and John T. Shepherd

ABSTRACT

Experiments were conducted in 35 anesthetized dogs to study the circulatory adjustments, after hemorrhage, mediated by vagal afferents from the cardiopulmonary area. With the aortic baroreceptors and chemoreceptors denervated and carotid baroreceptor and chemoreceptor activity held constant, hemorrhage of 10% of blood volume caused an increase in heart rate and constriction of the resistance vessels of the hindlimb, renal, and mesenteric beds and of the splanchnic venous bed. Vagotomy abolished these responses to hemorrhage, resulting in a greater decrease in blood pressure. With the carotid baroreflex operative, the constriction of the renal and mesenteric resistance vessels and of the splanchnic venous bed were similar to that obtained with only the vagi operative, but the constriction of the hindlimb resistance vessels was much greater and aortic pressure was better maintained. It is concluded that receptors in the cardiopulmonary area, subserved by vagal afferents, exert their main influence on the splanchnic resistance and capacitance vessels and on the renal circulation, whereas the carotid baroreceptors predominantly affect the muscle vessels.

KEY WORDS low pressure receptors carotid baroreceptors aortic depressor nerves peripheral circulation control of blood pressure

The arterial baroreceptors play an important role in the reflex activation of the sympathetic nerves during hemorrhage, but less is known about the role of receptors subserved by vagal afferents from the cardiopulmonary area. In the cat and the rabbit, cardiac afferent nerves have been shown to have an important inhibitory effect on the circulation (1-3). In the unanesthetized rabbit with sectioned aortic and carotid sinus nerves, hemorrhage still produces vasoconstriction in the kidney when the vagi are intact (4). Oberg and White (5) recently reported that afferent cardiac nerves in the cat could elicit tachycardia and vasoconstriction during blood loss, the latter being greater in the renal than in the muscle vessels.

In the dog, most studies of the reflexes originating in the heart and lungs have been performed with chemical stimuli or mechanical increases in pressure in the different cardiac cavities. Only vasodilator responses could be demonstrated in these experiments (6-8). Obviously, these methods are inadequate to examine the full range of activity of the cardiopulmonary receptors. Study of these receptors is further complicated by the difficulty of identifying the aortic nerves in the dog and hence of achieving denervation of the aortic arch without interfering with vagal afferents from the heart and lungs. In the present experiments, the technique recently described by Edis and Shepherd (9) was used to denervate the aortic arch selectively, and this, combined with control of the pressure in the carotid sinuses, permitted an examination of the role of afferents from regions other than the arterial baroreceptors and chemoreceptors in the circulatory adjustments to hemorrhage in the dog.

Methods

PREPARATION

Thirty-five dogs weighing 15-25 kg were anesthetized with thiopental and chloralose (15 and 80 mg/kg body weight, respectively) and...
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artificially ventilated at 10–12 cycles/min. Additional doses of chloralose (10 mg/kg) were administered to maintain an even plane of anesthesia. Heparin (3 mg/kg) was given prior to cannulation of the vessels and hourly thereafter (1 mg/kg).

Blood samples were withdrawn periodically during each experiment for measurement of arterial PO₂, PCO₂, and pH. Arterial PO₂ was maintained above 250 mm Hg by ventilating with pure oxygen, PCO₂ was kept between 30 and 40 mm Hg by adjusting the tidal volume, and bicarbonate (7.58%) was infused as needed to maintain the pH between 7.30 and 7.40.

Aortic Nerve Section.—Using a dissection microscope, both aortic nerves were cut at their junction with the cranial laryngeal nerves (9). After section, the identity of the nerves was confirmed by electrically stimulating the cephalic ends or recording nerve activity from the caudal segments. In each dog, the increase in systemic arterial pressure with occlusion of both common carotid arteries was greater after both aortic nerves were sectioned.

Carotid Sinus Isolation.—Both carotid sinuses were prepared according to a modified Moissejeff technique (10), permitting their reversible isolation from the systemic circulation. The pressure within the sinuses was monitored and could be adjusted at will. The occipital arteries were ligated at their origin from the external carotid arteries to exclude the carotid chemoreceptors (11).

Hemorrhage.—A standard blood loss of 10% of the blood volume, assuming the total blood volume to be 90 ml/kg (12), was used. Blood was withdrawn at a constant speed of 1 or 5 ml/sec from the left common carotid artery through a cannula with a large bore. Then, 30–120 seconds were allowed for hemodynamic measurements before the blood was reinfused at the same speed; this short time was chosen to minimize tissue hypoxia and changes in circulating catecholamines. A heat exchanger on the reinfusion line ensured that the temperature of the blood remained at 37°C.

MEASUREMENTS

All pressures were measured with strain-gauge transducers (Statham P23De) and recorded on an ultraviolet Visicorder (Honeywell 1508).

Aortic Pressure and Heart Rate.—Pressure was measured through a catheter inserted in the right brachial artery. Heart rate was calculated from the electrocardiogram.

Resistance Vessels.—The hindlimb, small intestine, and kidney were used to study the reflex responses of the resistance vessels. Each bed was perfused at constant flow with a roller pump, using autologous blood from the arterial circulation. A depulsatior and heat exchanger were interposed in the perfusion line, and the temperature of the blood was maintained at 37°C. The perfusion pressure was measured just proximal to the point of insertion of the cannula into the artery. The pump speed was adjusted at the beginning of each experiment to provide a perfusion pressure similar to the mean aortic pressure. The changes in perfusion pressure reflected changes in vascular resistance.

The hindlimb was perfused via the left external iliac artery with blood from the terminal aorta. To eliminate other sources of arterial inflow to the limb, all branches of the terminal aorta and the deep circumflex iliac and the deep caudal epigastric arteries were ligated. A segment of small intestine was vascularly isolated, and the mesenteric arterial branch to this segment was ligated. The left kidney was perfused with blood from the left common carotid artery. The renal cannula was placed in the abdominal aorta distal to the renal arteries. After perfusion was started, the aorta was clamped just above the left renal artery so that blood flow to the kidney was never interrupted, and all branches from the excluded aortic segment were ligated except for the left renal artery. Thus, all blood perfusing the kidney was delivered by the pump. A bypass line was introduced distally in the aorta to maintain arterial supply to the hindlimbs.

In some experiments, a donor dog was used to perfuse the left kidney at constant pressure, and renal blood flow was measured with a cannulating electromagnetic flowmeter (Carolina Medical Electronics) in the arterial line. The same method of arterial cannulation described previously was used. The left renal vein was also cannulated to return the venous blood to the donor. Zero flow was verified at regular intervals during which the blood flow was maintained through a bypass line. The perfusion pressure was that of the donor animal and was monitored in the perfusion line.

Capacitance Beds.—A cutaneous vein, the right lateral saphenous vein, was cannulated at the ankle and perfused at constant flow with a roller pump, using autologous blood from the terminal aorta, according to the technique described by Webb-Peploe and Shepherd (13). The speed of the pump was adjusted to obtain a perfusion pressure between 10 and 30 mm Hg. Gallamine (3 mg/kg) was given to prevent changes in venous pressure due to contractions of skeletal muscle. Under these conditions, the driving pressure—that is, the difference between perfusion (inflow) and femoral vein (outflow) pressures—was a measure of tone in the vein wall. The reactivity of the vein was assessed by cooling the perfusate from 37° to 27°C (14) at
the beginning of each experiment, immediately after the vagotomy, and at the end of the study. Cooling always induced a marked and rapid venoconstriction.

The splanchnic capacitance bed was studied by using the isovolumetric spleen technique. Splenic venous pressure was measured by a small catheter inserted in a hilar vein until its tip was in the hilum of the spleen. The spleen was vascularity isolated from the systemic circulation by ligating all vessels except for the splenic artery and vein. Snares were placed around the splenic vessels and occluded at the time observations were to be made. The blood volume in the spleen thus remained constant during the period of observation, and changes in venous pressure were caused by changes in tone of the splenic capsule and veins (15).

**Results**

Each dog was subjected to hemorrhage under the following conditions and in the order listed: (1) Carotid sinuses included in the systemic circulation, vagi intact, aortic nerves cut; (2) Carotid sinuses excluded from the systemic circulation and maintained at constant pressure, vagi intact, aortic nerves cut; (3) Carotid sinuses excluded from the systemic circulation and maintained at constant pressure, vagi cut, aortic nerves cut; (4) Carotid sinuses included in the systemic circulation, vagi cut, aortic nerves cut.

When the carotid baroreceptors participated in the reflex circulatory changes, the response to hemorrhage permitted an assessment of the reactivity of the preparation. It also provided some comparison of the response of the different vascular beds to hemorrhage when the carotid baroreflexes alone were operative with that when only the vagi were intact.

Primarily, this study was concerned with comparisons of reflex circulatory changes with the vagi intact as opposed to those with the vagi cut. Therefore, the aortic arch was denervated, and the carotid sinuses were excluded from the circulation and maintained at a constant static pressure. This pressure was adjusted to set the systemic arterial pressure at a value similar to that before the first hemorrhage; at least 5 minutes were allowed between the sinus pressure adjustments and the hemorrhages so that a steady state could be reached. Thus, there was no aortic baroreceptor or chemoreceptor activity, and the activity from the carotid baroreceptors and chemoreceptors was constant. The bilateral cervical vagotomy was done at the level of the cricoid cartilage.

The reflex reactivity of the animal was further checked before each hemorrhage by observing the response of aortic blood pressure to a sudden change in carotid sinus pressure from 200 to 40 mm Hg. Increased reflex response indicated the need for additional chloralose which was administered cautiously until the same degree of reactivity as before was obtained.

### Aortic Blood Pressure and Heart Rate

In eight dogs, only aortic blood pressure and heart rate were measured to determine the effect of hemorrhage in the absence of surgical interference with the cardiovascular system.

Efferent vagal activity was abolished by intravenous injection of atropine sulfate (0.2 mg/kg followed by 0.1 mg/kg hourly). Blood was withdrawn at a constant speed of 1 ml/sec and 30 seconds were allowed before reinfusion so that a steady state could be attained. Before bleeding, heart rate and mean aortic blood pressure were similar under each experimental condition. With vagal and carotid receptors operative, only vagal receptors operative, and only carotid receptors operative, hemorrhage caused the heart rate to increase by an average of 17, 10, and 15

<table>
<thead>
<tr>
<th>Baroreceptors operative</th>
<th>Mean aortic blood pressure (mm Hg)</th>
<th>Initial</th>
<th>Decrease</th>
<th>% decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vagal and carotid</td>
<td>128 ± 5</td>
<td>17 ± 2</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Vagal only</td>
<td>128 ± 5</td>
<td>31 ± 3*</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>126 ± 4</td>
<td>53 ± 3*</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Carotid only</td>
<td>125 ± 3</td>
<td>25 ± 3</td>
<td>18</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± s.e for eight dogs with both aortic nerves cut and after injection with atropine.

*Paired comparison shows difference to be statistically significant (P < 0.001).
Changes in hindlimb perfusion pressure (constant-flow perfusion) with 10% hemorrhage. Means from ten dogs with aortic nerves cut. Time scale is related to end of hemorrhage and reinfusion.

beats/min ($P < 0.05$), respectively. However, when no receptors were operative, there was no significant change in heart rate with hemorrhage (mean = 2 beats/min). The decreases in aortic pressure with hemorrhage in each experimental condition are shown in Table 1. With the carotid baroreceptor activity constant and the aortic arch denervated, the aortic pressure was better maintained during hemorrhage when the vagi were operative than after vagotomy. Comparison of the decreases in arterial pressure in these two conditions by the paired t-test showed that the difference was significant ($P < 0.001$).

**PERIPHERAL VASCULAR BEDS**

The reflex response of the peripheral beds to hemorrhage was studied in 27 dogs. To allow a longer period for hemodynamic stabilization without an increase in the duration of hypotension, the bleeding was at 5 ml/sec and completed in 30-60 seconds. The blood was reinfused at the same speed 2 minutes after the end of hemorrhage. In 10 dogs, changes in hindlimb vascular resistance and splenic capacitance were studied simultaneously; in 5 dogs, the responses of the lateral saphenous vein and the spleen were compared. The mesenteric resistance bed was investigated in 4 dogs and the renal resistance vessels in 8, five experiments being done with constant-flow perfusion of the kidney and three with constant-pressure perfusion.

In six experiments, right atrial pressure was measured, and the 10% hemorrhage resulted in an average decrease of 2.4 cm H$_2$O.

**RESISTANCE VESSELS**

**Hindlimb.**—In all ten animals, the perfusion pressure increased rapidly with hemorrhage when the carotid baroreflex was operative. This initial increase was followed by a decrease toward the control value. Reinfusion of the blood caused a sudden dilation before the pressure returned to the control level. With carotid baroreceptor activity constant and the vagi intact, there was a small and transient increase in pressure in eight dogs with bleeding; reinfusion of the blood also resulted in a transient vasodilation. When no receptors were operative, these responses to bleeding and reinfusion of the blood were absent in all but one dog (Fig. 1). The

<table>
<thead>
<tr>
<th>Baroreceptors</th>
<th>Hindlimb perfusion pressure (10)</th>
<th>Mesenteric artery perfusion pressure (4)</th>
<th>Splenic venous pressure (15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Max. Increase (mm Hg)</td>
<td>Initial</td>
</tr>
<tr>
<td>Vagal and carotid</td>
<td>164 ± 5</td>
<td>46 ± 9</td>
<td>174 ± 6</td>
</tr>
<tr>
<td>Vagal only</td>
<td>167 ± 7</td>
<td>11 ± 3</td>
<td>184 ± 8</td>
</tr>
<tr>
<td>None</td>
<td>172 ± 6</td>
<td>4 ± 3*</td>
<td>159 ± 10</td>
</tr>
<tr>
<td>Carotid only</td>
<td>159 ± 5</td>
<td>53 ± 9</td>
<td>161 ± 10</td>
</tr>
</tbody>
</table>

Data are means ± se from dogs with both aortic nerves cut. The number of dogs tested is given in parentheses.

*Not statistically significant ($P > 0.05$).
Changes in renal perfusion pressure (constant-flow perfusion) with 5% hemorrhage. Individual values before (B) and after (A) hemorrhage in five dogs with their aortic nerves cut. Operative baroreceptor areas are indicated.

maximal increases in perfusion pressure with hemorrhage in each of the conditions are shown in Table 2.

Kidney.—With constant-flow perfusion of the kidney, a 5% hemorrhage was sufficient to induce large increases in perfusion pressure when the carotid sinuses or only the vagi were operative (Fig. 2). The perfusion pressure gradually increased during the first minute after hemorrhage, sometimes by more than 100%. Twice, the blood had to be reinfused earlier because the pressure exceeded 300 mm Hg. When excessively high perfusion pressures were reached, a return to control could not be achieved with reinfusion and the pressure stabilized at a higher level. With carotid baroreceptor activity constant, vagotomy abolished the increase in pressure in four dogs and markedly decreased it in one dog. In two dogs, after denervation of the kidney, hemorrhage with the carotid sinuses operative produced no change in renal perfusion pressure, thus excluding a hormonal factor as the basis for the previous responses.

When the kidney was perfused at constant pressure, 5 and 10% hemorrhages performed successively resulted in a response of much lesser magnitude than at constant-flow perfusion (Fig. 3). With either carotid or vagal reflexes operative, the renal blood flow decreased immediately during hemorrhage and reached its lowest level by the end of the bleeding. Within the following minute, the flow had returned to or toward control value. On reinfusion of the blood, there was at first an increase in renal blood flow above the control level followed by a return to the control level. The maximal decrease in flow achieved with the 5% hemorrhage averaged 10% with the carotid baroreflex operative and the vagi intact, 9% with the vagi only, and 12% with the carotid baroreceptors alone. With the 10% hemorrhage, the flows were decreased by an average of 18, 17, and 18%, respectively. However, when no baroreceptor area was operative only minimal changes in flow were seen, averaging 5 and 4%, respectively, with 5 and 10% hemorrhages. After denervation of the kidney in two dogs, the 10% hemorrhage with the carotid baroreflex operative produced no changes in renal blood flow.

In some of these experiments, the dogs were permitted to breathe spontaneously, but the reflex response of the kidney vessels was in no way different from that observed with artificial respiration.

Splanchnic Vessels.—The perfusion pressure of the mesenteric artery increased by an average of 35 mm Hg after hemorrhage when the carotid sinuses were operative, both before and after vagotomy (Table 2). When the activity of the carotid sinuses was held constant, a similar average increase resulted with the vagi intact but was absent after vagotomy.
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FIGURE 4
Changes in venous pressure in isovolumetric spleen with 10% hemorrhage. Means from 15 dogs with their aortic nerves cut. Time scale is related to end of hemorrhage and reinfusion.

CAPACITANCE BEDS

Splenic Capacitance.—With the carotid baroreflex operative, both before and after vagotomy, hemorrhage resulted in an increase in splenic venous pressure in all dogs (Table 2). A similar increase was also present in each dog when only the vagi were intact. Maximal splenic response was reached at the end of the bleeding and was well maintained until the blood was reinfused. A rapid relaxation of the spleen followed reinfusion, the pressure returning to control values (Fig. 4). With carotid baroreceptor activity constant, vagotomy completely abolished the splenic response in seven dogs and markedly decreased it in seven others. In one dog, the splenic response persisted after vagotomy, along with the response of the hindlimb resistance vessels. Thus, as in the mesenteric resistance vessels, the response of the splenic capacitance vessels to hemorrhage with only vagal receptors operative was not significantly different from that with the carotid receptors operative.

A frequency-response curve was obtained for the spleen in two of these dogs by electrical stimulation of the splenic nerve with a supramaximal stimulus (25 v, 5 msec) at various frequencies of stimulation. The maximal splenic response was reached at a frequency of 4 cps in one and 10 cps in the other. The reflex response of the spleen to hemorrhage in the same dogs was equivalent to stimulation at a frequency of 1.5 and 5 cps, respectively—that is, to the midportion of each curve. Thus, the splenic response to hemorrhage was not limited by the maximal ability of the spleen to contract.

Hindlimb Cutaneous Vein.—Only small increases or decreases in the driving pressure in the saphenous vein occurred after hemorrhage in five dogs with vagal and carotid reflexes operative. With both vagal and carotid receptors operative, with only vagal, with neither, and with carotid alone, the mean (±SE) changes were decreases in pressure of 4 ± 3, 4 ± 3, 1 ± 1, and 0 ± 0.6 mm Hg, respectively. None of these changes was statistically significant, in contrast to the reflex constriction of the spleen measured simultaneously in these dogs. In each, the saphenous vein was very reactive to changes in temperature of the perfusate, constricting with cooling and dilating with warming (14). When the lumbar sympathetic chain on the side of the perfused saphenous vein was electrically stimulated (25 v, 5 msec) at the completion of two of these experiments, increases in perfusion pressure greater than 100 mm Hg were obtained with frequencies of 2 and 5 cps, respectively.

Discussion

In these experiments, carotid chemoreceptor activity was inhibited by ventilation with 100% oxygen and ligation of the occipital arteries at their origin. With both aortic nerves cut, the pressure in both carotid sinuses held constant, and the vagi intact, hemorrhage resulted in an increase in heart rate, an increase in resistance in the arterial beds of the hindlimb, kidney, and small intestine, and a constriction of the splenic capacitance elements. After vagotomy, these circulatory changes were abolished or markedly attenuated, indicating that they were caused by afferent information from the vagi. The vasoconstrictor responses were mediated by an increase in sympathetic nerve activity rather than by an increase in circulating catecholamines, because the renal responses to hemorrhage were absent after denervation of the kidney and the spleen was completely isolated from the circulation at the time of the experiments.
Previous studies have shown that sectioning of both aortic nerves effectively denervates the aortic baroreceptors and chemoreceptors in the dog (9). In addition, evidence has been presented that in this species the aortic baroreceptors, unlike the carotid, have little influence at normal or less than normal blood pressure (16). Therefore, the vascular reflex observed in the present experiment cannot have originated from the receptors of the aortic arch. Nor can splanchnic baroreceptors account for these hemodynamic effects, since Selkurt and Rothe (17) have shown that splanchnic baroreflex activity is slight and unaffected by vagotomy in the dog. Thus, receptors situated in the cardiopulmonary area must have been responsible for the vagal afferent information that resulted in the circulatory reflex responses to hemorrhage and hence the better maintenance of aortic blood pressure.

Our results are in agreement with the findings of Oberg and White (5) in the cat, showing that cardiac afferent nerves caused compensatory adjustments of the circulation after hemorrhage. In the dog, increases in neural traffic in cardiac afferent nerves, but not in the aortic nerves, have been reported with non-hypotensive hemorrhage (18). Evidence is also available to indicate that atrial receptors play an important role in the control of blood volume in the dog (19–21). Recently, Karim and associates (22) have demonstrated increases in cardiac and decreases in renal sympathetic nerve activity with balloon distention of the pulmonary vein-left atrial junction. However, our experiments do not provide information on the location in the cardiopulmonary area of the receptors involved in the reflex.

Extravagal pathways for afferent fibers have been described in rare instances (23). Such an aberrant pathway may account for the persistence of the vascular responses to hemorrhage in the one dog after vagotomy.

The main vascular effects of the vagal afferents were seen in the renal and splanchnic circulations. The responses of the resistance vessels of the kidney were strikingly different according to the technique of perfusion. While there were marked increases in renal perfusion pressure in the kidney perfused at constant flow, only modest and often transient decreases in renal blood flow were obtained with even larger hemorrhages in the kidney perfused at constant pressure. Haddy and associates (24) found that hemorrhage causes a much greater increase in renal vascular resistance when flow is held constant in the kidney than when it is permitted to decrease with the blood pressure. It is possible that autoregulation may be the cause of these different responses. The kidney participates in the arterial baroreflex (25–27), and from our results, there is no doubt that it also responds to the reflex subserved by vagal afferents. However, the magnitude of the responses cannot be assessed from the present experiments. Previous studies have shown that the changes in pressure in the isovolumetric spleen reflect the reflex response of the capacitance vessels throughout the splanchnic bed (15). Thus, the constriction of the splanchnic capacitance and resistance vessels caused by vagal afferents would permit active and passive mobilization of blood from the splanchnic area (28).

A comparison of the responses to hemorrhage in the condition in which the vagi alone were intact with that in which only the carotid sinuses were operative shows that the responses of the splanchnic resistance and capacitance vessels were similar, as were the changes in renal resistance. By contrast, the increase in hindlimb vascular resistance, while statistically significant with the vagi alone intact, was much greater when the carotid sinuses were operative. These findings suggest that the receptors subserved by vagal afferents have their main influence on the splanchnic bed and possibly on the kidney circulation, whereas the carotid sinus exerts its major effect on the muscle resistance vessels; this would account for the better maintenance of arterial blood pressure with the carotid sinus mechanism operative. Likewise, the aortic baroreceptors are known to influence preponderantly the muscle vascular bed, but their threshold in the dog is higher than that of the
carotid baroreceptors (16, 29). Thus, even if sectioning of both aortic nerves did not interrupt all the fibers from the aortic arch, this could not account for the differential pattern of reflex responses observed in these experiments.

The cutaneous veins do not react to hemorrhage, either via the carotid sinus mechanism or through vagal afferents. This is in agreement with previous studies showing that these vessels are mainly concerned with temperature regulation and do not participate in the baroreceptor reflexes (30).

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References

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