Effects of Hemorrhage on Renal Nerve Activity in Conscious Dogs

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SUMMARY. We studied the effects of slow continuous hemorrhage (0.5 ml/kg per min) on measurements of arterial and left atrial pressures, and renal nerve activity in conscious dogs with all reflexes intact, or after sinoaortic baroreceptor denervation, cardiac denervation, or sinoaortic baroreceptor denervation plus vagal denervation. In intact dogs, mean arterial pressure remained relatively constant at 101 ± 4 mm Hg until 20 ± 4 ml/kg of hemorrhage, when renal nerve activity increased by 211 ± 53%. At 39 ± 2 ml/kg hemorrhage, mean arterial pressure fell by 48 ± 3 mm Hg, and renal nerve activity returned to the prehemorrhage control level. Cardiac denervation did not affect the response of mean arterial pressure to hemorrhage, whereas, after sinoaortic baroreceptor denervation and sinoaortic baroreceptor plus vagal denervation, mean arterial pressure remained at its control level only through 8 ± 1 and 4 ± 1 ml/kg hemorrhage, respectively. The increases in renal nerve activity during nonhypotensive hemorrhage were significantly attenuated by either sinoaortic baroreceptor or cardiac denervation, and were completely blocked by sinoaortic baroreceptor plus vagal denervation. However, the decline in renal nerve activity with hypotensive hemorrhage was not blocked by either cardiac or sinoaortic baroreceptor denervation, and was enhanced after sinoaortic baroreceptor plus vagal denervation. Our data indicate that nonhypotensive hemorrhage in the conscious dog elicits a striking increase in renal nerve activity, which then returns to control levels during hypotensive hemorrhage. Both sinoaortic and cardiopulmonary baroreceptors are involved in mediating the increase in renal nerve activity, whereas the decline in renal nerve activity is not due to either of these baroreflexes.

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THE role of the cardiopulmonary and sinoaortic baroreceptors in the control of efferent renal sympathetic nerve activity during volume expansion has been a subject of considerable investigation (Clement et al., 1972; Thames et al., 1982; Morita and Vatner, 1985). In contrast, little is known about the response of renal nerve activity during volume depletion in the conscious animal, although Clement et al. (1972) have shown that renal nerve activity rises during hemorrhage in the anesthetized rabbit. Since anesthesia affects responses of systemic hemodynamics, renal blood flow, and vascular resistance to hemorrhage (Vatner, 1974; Gross et al., 1979, Zimpfer et al., 1982), it was considered important to determine the extent to which efferent sympathetic nerve activity to the kidney is increased in response to slow continuous hemorrhage in the conscious animal. In fact, a simple rise in renal nerve activity might not be predicted, since the conscious animal responds to moderate hemorrhage withrenal vasodilation (Vatner, 1974). To investigate the relative roles of cardiopulmonary and sinoaortic baroreceptors in the reflex changes in renal nerve activity during hemorrhage, we also studied groups of conscious dogs after sinoaortic baroreceptor denervation (SAD), cardiac denervation (CD), and SAD plus vagal denervation (VD).

Methods

All experiments were conducted in conscious dogs 2 weeks to 2 months after implantation of aortic and left atrial catheters, and 1-3 days after implantation of an electrode on the renal sympathetic nerves. The experiments in dogs with SAD or dogs with CD were conducted 2-3 weeks after denervation. Experiments in dogs with SAD plus VD were conducted 4-24 hours after vagotomy. The catheters were implanted under general anesthesia with sodium pentobarbital, 30 mg/kg (Lemmon Co), and at a subsequent operation, a recording electrode (Ninomiya et al., 1976) was implanted on the postganglionic renal nerves through either a right or left flank incision. Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Council [DHEW publication no. (NIH) 78-23, revised 1978].

For SAD and CD, the anesthetic used was sodium pentobarbital, 30 mg/kg, whereas, for VD, sodium thiamylal (Bio-Tal Biocut Laboratories), 10 mg/kg, was used. SAD was performed by bilateral cervical section of the carotid sinus nerves and stripping of the aortic arch, brachiocephalic, and subclavian arteries. CD was performed by the technique described by Randall et al. (1980). To obtain more complete deafferentation, we also stripped the adventitia from each of the pulmonary veins and the inferior vena cava at their junction with the pericardium.
and sectioned the ansa subclavae. Completeness of the CD was confirmed at surgery by direct electrical stimulation of the left and right thoracic vagi and ansa subclavae. The efficacy of the SAD and CD was confirmed in conscious dogs 2–3 weeks after operation by observing lack of reflex changes in heart rate in response to alterations in arterial pressure by intravenous injections of pharmacological agents (phenylephrine, Neosynephrine hydrochloride, Winthrop Laboratories, 10 µg/kg, and nitroglycerin, Lilly, 20 µg/kg). Phenylephrine increased mean arterial pressure by 61 mm Hg in SAD dogs and by 76 mm Hg in CD dogs, whereas nitroglycerin reduced mean arterial pressure by 59 mm Hg in SAD dogs and by 46 mm Hg in CD dogs. Moreover, in CD dogs, phenylephrine reduced renal nerve activity by 92% and nitroglycerin increased renal nerve activity by 118%, confirming that arterial baroreceptors were intact. However, in SAD dogs, renal nerve activity did not change with these doses of phenylephrine and nitroglycerin. With a larger dose of phenylephrine, which resulted in an increase in left atrial pressure, renal nerve activity decreased. Volume expansion with dextran in CD dogs failed to increase heart rate, but increased heart rate by 42 beats/min in SAD dogs, whereas left atrial injection of veratridine (0.2–0.8 µg/kg) reduced heart rate and mean arterial pressure by 23 beats/min and 35 mm Hg, respectively, in SAD dogs but not in CD dogs. Finally, when dogs were killed, tissue samples from the left ventricle were taken for tissue catecholamine measurements by means of the radioenzymatic method of DaPrada and Zurcher (1976). Tissue samples from CD hearts contained less than 2% of the catecholamine concentration found in intact dogs.

Arterial and left atrial pressures were sampled from the previously implanted heparin-filled Tygon catheters and measured with Statham P23ID (Statham Inst.) transducers. Electronic R-C filters with 2-second time constants were used to derive individual mean value measurements. Renal electroneurograms were recorded after amplifying the original renal nerve signal with a differential amplifier and using a bandpass filter of 30 Hz to 1 kHz (Grass p 511 with HIP 511 probe). The output from the amplifier was passed through a gate circuit for removing baseline noise, and the output from the gate circuit was rectified by an absolute value circuit and integrated by a resetting voltage integrator. Since the integration output is a function of renal nerve activity by area of pulses received and their frequency, the number of resets/min was taken as a measure of overall renal nerve activity. To quantify efferent renal nerve activity, the average values during the 30-minute control period before hemorrhage were defined as 100%. Renal nerve activity during hemorrhage was calculated as percent change from the control values.

After the conscious dogs had been monitored for 1–2 hours, a 30-minute control period was recorded and hemorrhage (0.5 ml/kg per min) was begun. Blood was withdrawn from a catheter in the inferior vena cava until a sustained fall in mean arterial pressure of 40–50 mm Hg was attained. All data were continuously measured and recorded on a multichannel analog tape recorder (model 8101, Honeywell) and were displayed on a multichannel oscillograph (model 200, Gould Inst.). All data were computed and stored in a Digital computer (PDP 11/34, Digital Equipment Corporation). Mean values ± SEM were calculated. All responses between groups were compared by analysis of variance (Armitage, 1974), while one response was compared to control values using Student’s t-test for paired comparisons.

Results

Although all variables were recorded continuously during hemorrhage, the data presented were collected at two points; the first, which is designated as nonhypotensive hemorrhage, occurred just before mean arterial pressure fell and was coincident with maximum increases in renal nerve activity; the second point was chosen after a sustained 40–50 mm Hg reduction in arterial pressure and is designated hypotensive hemorrhage. The mean data from each group measured at the prehemorrhage control period, nonhypotensive period, and hypotensive period are summarized in Table 1.

Figure 1 illustrates a typical response to hemorrhage in an intact, conscious dog. In intact dogs, mean arterial pressure remained relatively constant at 101 ± 4 mm Hg until 20 ± 4 ml/kg of hemorrhage. During hypotensive hemorrhage (39 ± 2 ml/kg blood loss), mean arterial pressure was decreased by 48 ± 3 mm Hg. CD had no influence on maintenance of mean arterial pressure during hemorrhage. After SAD or SAD plus VD, mean arterial pressure started to decrease earlier (P < 0.01) than in intact dogs, i.e., mean arterial pressure was maintained only through 8 ± 1 ml/kg and 4 ± 1 ml/kg hemorrhage, respectively, in these two groups (Table 1).

Heart rate increased during nonhypotensive hemorrhage in intact dogs; however, this reflex increase in heart rate was significantly attenuated or completely eliminated by SAD, CD, or SAD plus VD (P < 0.01) (Table 1). In intact dogs, during nonhypotensive hemorrhage, heart rate increased by 56 ± 14 beats/min, and then declined slightly during hypotensive hemorrhage to a value 25 ± 9 beats/min above control levels. In dogs with SAD, nonhypotensive hemorrhage did not alter heart rate, whereas hypotensive hemorrhage decreased heart rate by 14 ± 5 beats/min (P < 0.03). This decline in heart rate was completely eliminated by either CD or SAD plus VD.

In intact dogs, renal nerve activity increased gradually and reached its peak during nonhypotensive hemorrhage (+211 ± 53%, P < 0.03) (Table 1). However, when mean arterial pressure started to decrease, renal nerve activity also started to fall. During hypotensive hemorrhage, renal nerve activity was not different from its control level. In one intact dog studied under anesthesia immediately after the renal nerve electrode was implanted, renal nerve activity rose during hemorrhage and did not fall during hypotensive hemorrhage. After SAD or CD, the peak increases in renal nerve activity were significantly attenuated (P < 0.01). After SAD plus VD, the increase in renal nerve activity during nonhypotensive hemorrhage was abolished (Fig. 2). The decline in renal nerve activity associated with hypotension was still present after either SAD or CD. However, after SAD plus VD, the decline in renal nerve activity associated with hypotension was actually enhanced (Table 1).
To demonstrate that the decline in RNA with hypotensive hemorrhage in dogs with SAD and VD was not due to a deteriorating renal nerve preparation, the shed blood was reinfused, and hexamethonium (5 mg/kg) was administered. With reinfusion of shed blood, renal nerve activity returned to baseline levels. Ganglionic blockade then abolished renal nerve activity (Fig. 2).

**Discussion**

The results of the present investigation suggest that both sinoaortic and cardiopulmonary baroreceptors mediate the increases in renal nerve activity observed during nonhypotensive hemorrhage. It must be recognized that the term, nonhypotensive hemorrhage, refers to measurements of mean arterial pressure. Probably systolic arterial pressure and pulse pressure fell, resulting in unloading of arterial baroreceptors, even before mean arterial pressure fell. Furthermore, left atrial pressure fell before the reduction in mean arterial pressure (Fig. 1), which resulted in unloading of cardiopulmonary baroreflexes. The data demonstrating partial attenuation of reflex increases in renal nerve activity in dogs with SAD and dogs with CD further support the concept that both low pressure cardiopulmonary receptors and arterial baroreflexes contribute to the rise in renal nerve activity in response to hemorrhage, prior to the reduction in mean arterial pressure.

The finding that renal nerve activity decreased toward prehemorrhage control levels during hypotensive hemorrhage was unexpected, since sensory inputs from both sinoaortic and cardiopulmonary baroreceptors are unloaded at this time, which should act in concert to increase renal nerve activity (Gupta et al., 1966; Clement et al., 1972). The possibility of "baroreceptor resetting" was considered (Coleridge et al., 1981; Dorward et al., 1982; Unde-
FIGURE 1. An original record illustrating responses of phasic and mean arterial pressure (AP), renal electroneurograms (RNA), integrated RNA, and mean left atrial pressure (LAP), to slow continuous hemorrhage in an intact conscious dog. Records are shown during the control period and the beginning of hemorrhage (left panel), 10 ml/kg of hemorrhage (middle panel), and hypotensive hemorrhage (right panel). The arrow indicates the beginning of hemorrhage. Renal nerve activity with hypotensive hemorrhage is shown in the right panel.

FIGURE 2. An original record illustrating responses of phasic and mean arterial pressure (AP), renal electroneurograms (RNA), integrated RNA, and mean left atrial pressure (LAP), to slow continuous hemorrhage in a conscious dog with SAD and VD. Records are shown during control and the beginning of hemorrhage (panel 1), nonhypotensive hemorrhage (panel 2), hypotensive hemorrhage (panel 3), and after reinfusion of shed blood (panel 4). The arrow under the fourth panel indicates an injection of hexamethonium (5 mg/kg).

Mayser et al., 1984). However, resetting of arterial baroreceptors is not the explanation for the reduction in renal nerve activity, since the decrease was still observed with hemorrhage in dogs with SAD. Activation of vagal C-fibers is another potential mechanism of the reduction in renal nerve activity. Excitation of ventricular receptors with nonmyelinated vagal fibers has been shown to inhibit the vasomotor center, resulting in bradycardia (Oberg and Thoren, 1972) and a fall in arterial pressure (Oberg and
White, 1970). These receptors are excited primarily by ventricular distension, but also are excited when diastolic filling is low and sympathetic stimulation to the heart is high (Oberg and Thoren, 1972). Increased vagal restraint also has been observed by Chen et al. (1978) and Ebert et al. (1962) in response to hypotension. These vagal mechanisms could explain the reduction in heart rate with hemorrhagic hypotension, since this was blocked by CD or SAD plus VD, but not by SAD alone. However, these vagal mechanisms could not account for the decline in renal nerve activity with hemorrhagic hypotension, since neither CD nor SAD plus VD blocked the reduction in renal nerve activity and, in fact, the greatest reduction in renal nerve activity below control levels occurred in dogs with SAD plus VD.

If sympathetic afferents are not only excitatory but also exhibit resting tone, then these reflexes might be considered as the mechanism for the reduction in renal nerve activity with hypotensive hemorrhage. However, previous studies on responses to gradual hemorrhage have not demonstrated reduced firing of sympathetic afferents (Bishop et al., 1983). Furthermore, since total cardiac denervation failed to prevent the decline in renal nerve activity with hypotensive hemorrhage in the present investigation, it is unlikely that the stimulus for this response arose from cardiac sympathetic afferents.

Finally, the reduction in renal nerve activity with hypotensive hemorrhage was not due to the deterioration in the condition of the renal nerves, because, after reinfusion of shed blood, renal nerve activity gradually recovered to the prehemorrhage level (Fig. 2).

A previous study in conscious dogs demonstrated that hemorrhage elicits renal vasodilation (Vatner, 1974). The renal vasodilation was most prominent when arterial pressure fell and can be ascribed to an autoregulatory mechanism (Vatner, 1974; Gross et al., 1979). It is interesting that in the present study, renal nerve activity also fell during hypotensive hemorrhage, which could have facilitated the renal vasodilation. Thus, the renal vasodilation during hemorrhage observed in earlier studies is less surprising, when one recognizes that renal nerve activity does not remain at elevated levels with hemorrhage in the conscious dog. The prior study by Clement et al. (1972) in anesthetized rabbits, and the one anesthetized dog included in the present study, did not demonstrate a fall in renal nerve activity during hypotensive hemorrhage. Thus, the response of renal nerve activity may be quite different in conscious and anesthetized animals and may explain why differences have been observed in responses of renal blood flow and vascular resistance to hemorrhage in conscious and anesthetized animals (Vatner, 1974; Zimpfer et al., 1982).

In summary, in conscious dogs, nonhypotensive hemorrhage elicits increases in renal nerve activity, mediated by both sinoaortic and cardiopulmonary receptors. During hypotensive hemorrhage, renal nerve activity starts to decrease. This decrease is due to neither of these baroreflexes. This decline in renal nerve activity during hypotensive hemorrhage is not consistent with the concept that hemorrhagic hypotension elicits a sustained augmentation of sympathetic drive to the entire cardiovascular system of the conscious animal.

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