Preoptic Hypothalamic Control of Arteriolar Vasodilatory Responses

Steven L. Bealer and Kenneth G. Proctor

Arteriolar responses to hemorrhage were directly observed in the skeletal muscle and intestinal circulations of two groups of animals. In one group, the periventricular tissue surrounding the anteroventral portion of the third cerebral ventricle (AV3V) was electrolytically lesioned 7–10 days before an acute experiment. In the other group, control surgical procedures were performed. Hemorrhage resulted in similar decreases in arterial blood pressure (30–60% prehemorrhage level) and intestinal blood flow (20–60% prehemorrhage level) in both groups. In contrast, arteriolar diameter significantly increased following hemorrhage in the spinotrapezius muscle of control-operated animals (141±9% prehemorrhage level) but did not change in animals with AV3V lesions (97±7% prehemorrhage level). In a previous study, electrical stimulation of intact AV3V (issue was shown to cause a sustained decrease in blood pressure (80–90% of control level) and a vasodilation in skeletal muscle arterioles (120–140% control level). Since stimulation of intact AV3V tissue evokes vasodilation and AV3V tissue ablation reduces hemorrhage-induced vasodilation, this region of the hypothalamus may play an important role in the regulation or modulation of some responses in the peripheral microcirculation. (Circulation Research 1987;61(suppl II):II-32-II-35)

The periventricular tissue surrounding the preoptic recess in the anteroventral-most portion of the third cerebral ventricle (the AV3V region) is critical for pressor responses to several hormonal stimuli and for development of some forms of experimental hypertension and may play a role in survival following hemorrhage. In addition, electrical stimulation of this brain region reduces arterial blood pressure, alters resistance to blood flow in the hindquarters and the renal and mesenteric vascular beds, and results in arteriolar vasodilation of the spinotrapezius muscle.

The purpose of the present study was to determine further the role of the AV3V region in regulation of the peripheral microcirculation. Arteriolar responses in two different vascular beds were directly observed prior to and following hemorrhage in animals that had received electrolytic ablation of the AV3V region or had undergone control surgical procedures. Skeletal muscle and intestine were selected for study because these two regional circulations are particularly important in overall cardiovascular homeostasis.

Materials and Methods

Male Sprague-Dawley rats (160–250 g) were housed in a room maintained at 22° C and kept on a 12-hour light/dark cycle with ad libitum access to food and water. Animals were anesthetized with Brevital (50 mg/kg), placed in a stereotaxic instrument, and received electrolytic lesions in the AV3V region using procedures described previously. Briefly, the tip of a 24-gauge stainless steel lesioning electrode, insulated except for 0.5 mm at the tip, was placed in the AV3V region (–0.1 mm posterior to bregma, 0.0 mm lateral to the midline, and 7.3 mm ventral to the dura mater). An anodal lesion was produced by passing a 3-mA current through the electrode for 10–15 seconds.

Rats that received control operations were treated similarly, except the electrode was placed only 5.3 mm ventral to the surface of the brain, and no current was passed. All animals were allowed 10–14 days to recover before testing.

Preparation of the Microcirculation

Spinotrapezius muscle. The spinotrapezius muscle was prepared as described previously. Rats were anesthetized with an aqueous mixture of 1% a-chloralose and 13% urethane (1.2 ml/100 g i.p.) and were given supplemental doses as needed (0.1–0.3 ml/100 g i.v.). Body temperature was continuously monitored and maintained at 36–38° C using a heat lamp. The trachea and both femoral arteries were catheterized. The rats breathed spontaneously on room air. Femoral arterial blood pressure was monitored with a Statham P23Db transducer and a Gould physiologic recorder (Oxnard, Calif.). Supplemental anesthetic and fluids were administered through a catheter in the jugular vein.

A midline, longitudinal incision was made in the back of the animal, and the skin was removed to expose the spinotrapezius muscle. The muscle was gently dissected free from adhering connective tissue while being continuously superfused with bicarbonate-buffered (20 mM) Ringer's solution maintained at 36–38° C, pH 7.35–7.45. The tissue was gently reflected over a glass viewing port and transilluminated with filtered white light (540 nm). The microcirculation was viewed using a long-working-distance condenser.
(0.45 numerical aperture; Leitz L-20, Rockleigh, N.J.) and objective (0.4 numerical aperture; Leitz L-32) and a microscope with a trinocular head (Leitz Laborlux II), a Phillips video camera (Mahwah, N.J.) conveyed the image to a Colorado Model 321 video analyzer, For-A Model VTVG-33 video timer (Tokyo), and Sony monitor. The analog output of the video analyzer, modified to perform as a video micrometer, was used to measure arteriolar diameter. This system has a reproducibility of better than 1.0 μm.14

SMALL INTESTINE. The intestine was prepared using a modification15,16 of a technique originally described by Bohlen and Gore.17 The mucosal and muscularis surfaces were suffused with bicarbonate-buffered Ringer’s solution maintained at 36–38° C. Isoproterenol (0.05 μM; Sigma Chemical Co., St. Louis, Mo.) was added to the serosal suffusate to suppress spontaneous motility. The threshold concentration of isoproterenol required to produce vasodilation is 1 μM.15

The intestinal microcirculation was transilluminated and observed using optical equipment and techniques similar to those described for the spinotrapezius muscle. In addition to arterial blood pressure and arteriolar diameter, red cell velocity was measured in the intestine using a rotating prism velocimeter.15 Intestinal blood flow was calculated from the product of a constant, arteriolar diameter, and red cell velocity.15

Protocol

AV3V ABLATION AND HEMORRHAGE. Responses in the microcirculation of the spinotrapezius muscle and the intestine were observed in separate groups of AV3V-lesioned and control-operated animals.

After surgical preparation, the small intestine was allowed 30–60 minutes to stabilize prior to testing. Vasomotor tone in a first-order arteriole17 in the submucosa was tested by topical application of 500 μM adenosine to the serosa. The experiment was terminated if topical adenosine failed to cause transient vasodilation.

After a 10–30-minute steady-state baseline, the animals were hemorrhaged (volume = 1.0% body weight, withdrawn over 1 minute). Blood pressure was continuously measured from one femoral artery catheter while blood was withdrawn from the contralateral artery. All hemodynamic measures were monitored until new steady-state levels were attained.

The identical protocol was repeated in separate groups of animals to study the spinotrapezius muscle microcirculation.

Histology

After a successful acute experiment, rats were transcardially perfused with 0.9% saline followed by phosphate-buffered formalin solution. The brains were removed and stored until processing for histologic verification of lesion location. The tissue block containing the AV3V region was frozen, cut in 40-μm sections, mounted on albumin-coated slides, and stained with cresyl-violet. The position and extent of tissue damage was evaluated under the light microscope.

Statistics

Data are represented as mean ± SEM. Statistical differences were analyzed using within-group analysis of variance with repeated measures and a posteriori analysis for differences between individual means (Newman-Keuls) or Student’s t test where appropriate. Between-group differences were evaluated using analysis of variance and t test where appropriate.

Results

Figure 1 shows the effect of hemorrhage on blood pressure (top panel) and arteriolar diameter (bottom panel) in the spinotrapezius muscle of control-operated rats (solid circles) and rats with AV3V lesions (open circles). There were no significant differences in resting blood pressure or arteriolar diameter prior to hemorrhage between the two groups of rats. Hemorrhage produced significant decreases in blood pressure within both groups, but there was no difference between the groups. Arteriolar diameter significantly increased for 1–2 minutes following hemorrhage in control-operated rats and thereafter stabilized near the baseline level. However, arteriolar diameter in rats with AV3V lesions was not significantly different than prehemorrhage baseline levels at any time during the protocol. These observations suggest that intact AV3V tissue is important for the transient vasodilatory response in skeletal muscle following hemorrhage.

Figure 2 illustrates the responses observed in the intestinal microcirculation following hemorrhage. These groups of rats also showed similar, significant decreases in arterial blood pressure following hemorrhage (top panel) that were not significantly different than blood pressure values in Figure 1. The middle panel of Figure 2 shows that there was no significant change in arteriolar diameter in either group at any
time. As shown in the bottom pane (Figure 2), hemorrhage caused similar reductions in calculated blood flow in the intestinal microcirculation in both groups. These observations suggest that the AV3V region does not exert measurable control of the intestinal microcirculation during hemorrhage.

A corollary to the data shown in Figures 1 and 2 is that the skeletal muscle circulation may be tonically influenced by the AV3V region, while the intestinal circulation is primarily influenced by other neurohumoral mechanisms during hemorrhage.

The lesions produced in these rats were similar to those described in previous studies by this laboratory and by others. Lesion damage was restricted to the anterior wall of the third cerebral ventricle, with bilateral ependymal and periependymal damage extending from the lamina terminalis to approximately the border of the medial preoptic area and the anterior hypothalamic nucleus.

Discussion

These data have demonstrated that electrolytic ablation of the AV3V region abolishes the transient arteriolar vasodilation evoked in the spinotrapezius muscle following hemorrhage. However, microvascular responses to hemorrhage in the intestinal microcirculation were similar between control-operated rats and rats with AV3V lesions. These data suggest that this brain region may be important in controlling microcirculatory hemodynamics in skeletal muscle during hemorrhage.

Previous studies have shown that electrical stimulation of intact AV3V tissue results in increased arteriolar diameter and a depressor response. Results from the present experiments suggest that skeletal muscle vasodilation associated with AV3V stimulation cannot be considered a simple autoregulatory response since AV3V ablation abolished vasodilation during hemorrhage-induced hypotension (Figure 1). In conjunction with our earlier study, these data suggest that this brain region plays an active role in regulation of vasodilation within the peripheral circulation.

The mechanism that accounts for the abolition of the hemorrhage-induced vasodilation in the muscle of animals with AV3V lesions has not been determined. It is well known that arteriolar diameter reflects a balance of neurogenic, hormonal, and local factors. Autoregulatory vasodilation usually accompanies severe hypotension in most experiment conditions. This response is locally mediated by a myogenic reflex and by accumulation of vasodilatory metabolites that initially predominate over neural and hormonal vasoconstrictor influences.

One possible explanation for the abolition of the hemorrhage-induced vasodilation in rats with AV3V lesions may be that ablation of this brain region produces a chronic increase in vasoconstrictor tone that counteracts the local vasodilatory influences during hypotension. The vasoconstrictor mechanism responsible for increased arteriolar tone is unknown. Ablation of AV3V tissue does not produce significant increases in circulating concentrations of catecholamines or vasopressin. However, plasma renin concentration is significantly elevated following ablation of this brain region. Consequently, angiotensin II may contribute to increased vasoconstrictor tone in rats with AV3V lesions.

An alternative explanation for the loss of vasodilatory responses in rats with AV3V lesions may be elimination of a vasodilator system normally activated by hemorrhage. Determining the precise mechanism by which the AV3V region controls vasoconstrictor tone and vasodilatory responses requires further investigation.

The periventricular tissue surrounding the AV3V has been shown to have profound effects on cardiovascular regulation. Ablation of this region results in both acute and chronic alterations in cardiovascular responses. Immediately following AV3V ablation, rats exhibit reduced plasma and blood volumes and transient hypertension. Within 24 hours following ablation, arterial blood pressure returns to baseline levels. Although rats with AV3V lesions are normotensive, they continue to have attenuated pressor responses to a number of stimuli and chronically decreased plasma volume. Furthermore, AV3V ablation prevents or attenuates the development of several forms of experimental hypertension, and rats with AV3V lesions do
not survive hemorrhage as well as control-operated rats. Other studies have attempted to define the role of this brain region in cardiovascular regulation by observing responses induced by electrical stimulation of intact AV3V tissue. Results from these experiments have shown that electrical stimulation of the AV3V evokes a depressor response and an alteration in peripheral blood flow.

Since changes in microcirculatory hemodynamics have been implicated in hypertension, plasma volume regulation, control of regional blood flow, and recovery of plasma volume following hemorrhage, it is reasonable to conclude that the AV3V region may modulate microvascular responses in the peripheral circulation. This hypothesis is supported by the results from the present study. However, further studies are needed to determine the precise mechanisms and physiologic significance of AV3V modulation of microvascular responses.

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References

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