Evidence that hemorrhagic hypotension is mediated by the ventrolateral periaqueductal gray region

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Cavun, Sinan, and William R. Millington. Evidence that hemorrhagic hypotension is mediated by the ventrolateral periaqueductal gray region. Am J Physiol Regulatory Integrative Comp Physiol 281: R747–R752, 2001.—Severe hemorrhage lowers arterial pressure by suppressing sympathetic activity. This study tested the hypothesis that the decompensatory phase of hemorrhage is mediated by the ventrolateral periaqueductal gray (vlPAG), a region importantly involved in the autonomic and behavioral responses to stress and trauma. Neuronal activity in the vlPAG was inhibited with either lidocaine or cobalt chloride 5 min before hemorrhage (2.5 ml/100 g body wt) was initiated in conscious, unrestrained rats. Bilateral injection of lidocaine (0.5 µl of a 2% or 1 µl of a 5% solution) into the caudal vlPAG delayed the onset and reduced the magnitude of the hypotension produced by hemorrhage significantly. In contrast, inactivation of the dorsolateral PAG with lidocaine was ineffective. Cobalt chloride (5 mM; 0.5 µl), which inhibits synaptic transmission but not axonal conductance, also attenuated hemorrhagic hypotension significantly. Microinjection of lidocaine or cobalt chloride into the vlPAG of normotensive, nonhemorrhaged rats did not influence cardiovascular function. These data indicate that the vlPAG plays an important role in the response to hemorrhage.

SEVERE HEMORRHAGE lowers arterial pressure through a central mechanism (37). During progressive hemorrhage, arterial pressure is initially maintained within normal limits by a compensatory increase in sympathetic activity, but if allowed to progress, a second, decompensatory phase develops in which sympathetic activity abruptly decreases and blood pressure falls precipitously (4, 28, 37–39). The central mechanism that initiates the decompensatory phase of hemorrhage and thus lowers arterial pressure is not fully understood.

This study tested the hypothesis that the ventrolateral midbrain periaqueductal gray region (vlPAG) plays an important role in triggering the decompensatory phase of hemorrhage. The PAG is a functionally heterogeneous region that is thought to coordinate the autonomic, behavioral, and antinociceptive reactions to severe stress and injury (1, 2, 27). The PAG is organized into functionally and histologically distinct longitudinal columns. Activation of neurons in the lateral and dorsolateral PAG (dlPAG) columns with excitatory amino acids produces hypertension, tachycardia, sympathetic activation, nonopioid antinociception, and a behavioral syndrome characterized by hyperactivity, vocalization, and aggressive or escape behaviors: a typical “fight or flight” response. Activation of the vlPAG column, however, evokes a strikingly different response, characterized by an abrupt fall in arterial pressure, heart rate, and sympathetic activity, opioid-dependent antinociception, and behavioral quiescence and hyporeactivity. This syndrome is often likened to a predator defense reaction or to the hypoactivity that follows serious injury, visceral pain, or intense physical exercise (1, 2, 27).

The concept that vlPAG neurons mediate the response to severe pain and injury is supported by evidence that deep somatic or visceral pain and tissue injury stimulate expression of the intermediate/early gene c-fos in vlPAG neurons selectively (9, 21). Visceral pain also evokes an abrupt fall in arterial pressure and behavioral quiescence, a syndrome much like that caused by activation of vlPAG neurons with excitatory amino acids (1, 9). The hypotension produced by visceral pain (9) or excitatory amino acid injection into the vlPAG (6, 22) is relatively mild, however, generally on the order of 10–20 mmHg. It is not known whether the vlPAG is also capable of triggering the fall in arterial pressure caused by severe blood loss. To test this, we inhibited neuronal activity in the vlPAG of conscious, unrestrained rats with either lidocaine or cobalt chloride before initiating hemorrhage.

METHODS

Forty-nine male Sprague-Dawley rats (250–300 g; Taconic Farms, Germantown, NY) were anesthetized with halothane (1.5–4% in 100% O₂), the left carotid artery was cannulated with PE-50 tubing filled with heparinized saline (100 U/ml), and the cannula was exteriorized at the nape of the neck and sealed until use. To inject lidocaine or cobalt chloride into the vlPAG, two 26-gauge stainless steel guide cannulas were placed bilaterally into the lateral midbrain periaqueductal gray region (vlPAG) of restrained rats. Bilateral injection of lidocaine (0.5 ml of a 5% solution) into the caudal vlPAG delayed the onset and reduced the magnitude of the hypotension produced by hemorrhage significantly. In contrast, inactivation of the dorsolateral PAG with lidocaine was ineffective. Cobalt chloride (5 mM; 0.5 µl), which inhibits synaptic transmission but not axonal conductance, also attenuated hemorrhagic hypotension significantly. Microinjection of lidocaine or cobalt chloride into the vlPAG of normotensive, nonhemorrhaged rats did not influence cardiovascular function. These data indicate that the vlPAG plays an important role in the response to hemorrhage.

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inserted at a 27° rostral-caudal angle through burr holes drilled bilaterally through the skull. The tips of the guide cannulas were positioned 0.8 mm lateral and 8.3 mm posterior to bregma at a depth of 6.2 mm below the skull surface according to the atlas of Paxinos and Watson (33). For dIPAG injections, the guide cannulas were implanted bilaterally 0.8 mm lateral and 8.3 mm posterior to bregma and 4.6 mm below the skull surface.

Four to six hours after the animals recovered from anesthesia, the arterial catheter was attached to a volumetric pressure transducer, and arterial pressure and heart rate were recorded at 1-min intervals using a MicroMed BPA-200 blood pressure analyzer (Micro-Med, Louisville, KY). After stable baseline blood pressure and heart rate recordings were obtained, two 33-gauge injection cannulas were inserted through the guide cannulas, and lidocaine (0.5 μl of a 2% or 1.0 μl of a 5% solution; Sigma Chemical, St. Louis, MO), cobalt chloride (0.5 μl of a 5 mM solution; Sigma Chemical), or saline was injected through each cannula at a constant rate of 0.5 μl/min. The injection volume was monitored by observing the movement of an air bubble placed in the tubing. Five minutes later, hemorrhage was initiated by disconnecting the arterial cannula from the pressure transducer and allowing blood (2.5 ml/100 g body wt) to flow through the carotid artery cannula at a controlled and relatively constant rate of 0.3–0.4 ml/min for 20 min (32). Blood loss was stopped briefly at 5-min intervals to record arterial pressure and heart rate. After the hemorrhage period, arterial pressure and heart rate were recorded at 1-min intervals and reported and analyzed at 5-min intervals for 60 min. Each animal was used in only one experiment. At the end of each experiment, the injection sites were marked with 0.5 μl India ink, and the brain was removed, immersed in 10% paraformaldehyde, sectioned, and stained with eosin.

Paired data were analyzed by two-tailed Student’s t-test, and multiple comparisons were analyzed by repeated-measures analysis of variance followed by Dunnett’s multiple comparisons test. The surgical and experimental protocols were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

RESULTS

During progressive hemorrhage, mean arterial pressure was initially sustained at baseline levels (123.4 ± 1.2 mmHg) in saline-treated control animals, but after 5 min, it began to decline and reached its lowest point (49.6 ± 4.6 mmHg) at the end of the 20-min hemorrhage period (Fig. 1). Heart rate did not change during blood withdrawal and was not significantly different from initial baseline values (387 ± 10 beats/min) when hemorrhage was terminated (388 ± 23 beats/min) (Fig. 1).

Bilateral lidocaine (0.5 μl of a 2% solution) injection into the caudal viPAG inhibited hemorrhagic hypotension significantly (Fig. 1). Lidocaine pretreatment also delayed the onset of the hypotensive phase of hemorrhage (Fig. 1). Analysis of variance confirmed that lidocaine produced significant treatment [F(3,19) = 256, P < 0.001], time [F(16,323) = 43.2, P < 0.001], and treatment-time interaction [F(48,323) = 5.9, P < 0.001] effects. A higher lidocaine concentration (5%) and injection volume (1.0 μl) also reduced the magnitude and delayed the onset of hemorrhage-induced hypotension, although the response was not significantly greater than that produced by 0.5 μl of 2% lidocaine (Fig. 1). Lidocaine pretreatment also produced a small increase in heart rate in hemorrhaged animals. Analysis of variance demonstrated that lidocaine produced a significant treatment effect on heart rate [F(3,16) = 28.0, P < 0.001], but time and treatment-time effects were not significant; post hoc analysis showed that 5% lidocaine significantly increased heart rate, but 2% lidocaine did not. In control exper-
iments with nonhemorrhaged animals, vlPAG lidoca-
aine administration did not change mean arterial
pressure or heart rate (Fig. 1); saline injection also had
no effect on cardiovascular function (data not shown).
These data indicate that lidocaine inactivation of the
caudal vlPAG inhibits the decompensatory phase of
hemorrhage but does not influence cardiovascular ho-
meostasis in normotensive animals.

A notable disadvantage of lidocaine is that it blocks
axonal conduction in fibers of passage (30). To over-
come this liability, we tested whether hemorrhagic
hypotension is attenuated by cobalt chloride, which
blocks synaptic transmission without inhibiting axonal
conduction. Cobalt chloride (0.5 μl of a 5 mM solution)
injection into the vlPAG bilaterally inhibited the fall in
arterial pressure caused by hemorrhage significantly
(Fig. 2). Analysis of variance confirmed that cobalt
chloride treatment delayed the onset and duration of
hemorrhagic hypotension. Cobalt chloride injection produced an even
greater inhibitory response, indicating that hemor-
raghic hypotension is mediated by synaptic activity
within the vlPAG rather than by fibers of passage. In
contrast, inactivation of the dlPAG was ineffective.

Together, these data indicate that hemorrhagic hypo-
tension is mediated, at least in part, by the vlPAG, but
not the dlPAG.

DISCUSSION

Activation of vlPAG neurons with excitatory amino
acids evokes a coordinated series of autonomic
and behavioral reactions strikingly similar to the response
to deep tissue injury (9, 13, 21) or severe blood loss.
Conversely, vlPAG neurons are preferentially ac-

Fig. 2. Cobalt chloride injection into the vlPAG inhibits hemorrhagic
hypotension. Cobalt chloride (0.5 μl of a 5 mM solution) or saline was
injected bilaterally immediately before hemorrhage (2.5 ml/100 g
body wt) was initiated. Numbers in parentheses indicate the number
of animals in each group. Baseline MAP at the 25-min time point
was 122.5 ± 1.4 mmHg for the saline + hemorrhage group, 122.7 ±
2.0 mmHg for the cobalt chloride + hemorrhage group. Baseline
heart rate was 394 ± 17 beats/min for the saline + hemorrhage
group, 372 ± 14 beats/min for the cobalt chloride + hemorrhage
group, and 368 ± 15 beats/min for cobalt chloride-treated, nonhemor-
raghage controls. *P < 0.05, **P < 0.01 compared with the same time
point for saline-treated control animals.
rhaged rats, indicating that the vlPAG does not contribute substantially to the tonic regulation of cardiovascular homeostasis. These findings support the hypothesis that the vlPAG plays an important role in the response to hemorrhage.

Although 2% lidocaine injection into the vlPAG reduced the fall in arterial pressure caused by hemorrhage substantially, it did not prevent it completely. This did not result from an insufficient lidocaine dose because a larger lidocaine concentration (5%) did not produce a significantly greater inhibitory effect, nor is it likely to have resulted from an insufficient duration of action because a second lidocaine injection at the midpoint of the hemorrhage period did not enhance its inhibitory potency (unpublished data). Previous studies have shown that lidocaine inhibits neuronal activity for up to 1 h after intracerebral injection (17, 24, 30, 36). Kirouac and Pittman (24) reported, for example, that injection of lidocaine or cobalt chloride into the vlPAG blocked the depressor response produced by activation of the ventral tegmental area for up to 45 and 25 min, respectively. Lidocaine’s inhibitory efficacy was also unrelated to hemorrhage severity to the extent that lidocaine administration produced a comparable response in rats subjected to mild hemorrhage (1.9 ml/100 g body wt) (unpublished data). A more plausible reason that lidocaine administration inhibits, but does not fully prevent, hemorrhage from lowering arterial pressure is that it inactivates only a small proportion of the vlPAG. A lidocaine injection volume of 0.5 μl is estimated to spread to a diameter of ~1 mm in brain tissue (30, 36) (unpublished data), whereas the vlPAG extends for several millimeters through the midbrain. Alternatively, the decompensatory phase of hemorrhage may be mediated by multiple anatomic pathways.

The concept that hemorrhagic hypotension is triggered by activation of vlPAG neurons is consistent with a report that hemorrhage induces c-fos expression by vlPAG neurons selectively (1). However, Fos expression alone is not sufficient to demonstrate that activation of vlPAG neurons actually causes the sympathoinhibitory phase of hemorrhage. Nitroprusside administration also induces c-fos in the vlPAG (31, 41), which suggests that c-fos induction could be either the cause or consequence of hypotension. Furthermore, because hemorrhage produces a biphasic effect on sympathetic outflow, initially increasing, then inhibiting, sympathetic neuronal activity (37), it is possible that c-fos is expressed during the sympathoexcitatory, rather than the sympathoinhibitory, phase of hemorrhage.

An earlier report by Ward and Darlington (43) indicates that the vlPAG does, in fact, play a role in the initial sympathoexcitatory phase of hemorrhage. They
found that lesioning the caudal vlPAG essentially eliminated the compensatory increase in vascular resistance initially produced by hemorrhage in anesthetized cats without affecting resting arterial pressure or heart rate. Their report is the first, to our knowledge, to implicate the vlPAG in the response to hemorrhage. Their findings differ from the present data, however, which show that chemical inactivation of the vlPAG prolongs, rather than shortens, the compensatory phase of hemorrhage.

The vlPAG innervates a number of medullary cardioregulatory centers that influence the activity of sympathetic neurons, including the rostral ventrolateral medulla (RVLM) pressor region (23). Activation of vlPAG neurons inhibits the firing frequencies of spinally projecting RVLM neurons (26, 27), which suggests that the vlPAG may precipitate the compensatory phase of hemorrhage by directly inhibiting the RVLM. The vlPAG also innervates two medullary depressor sites in the caudal ventrolateral medulla (CVLM) and caudal midline medulla (CMM) (5, 8, 10, 16). The CVLM inhibits sympathetic outflow through an inhibitory projection to the RVLM and plays an important role in the baroreceptor-mediated regulation of cardiovascular function (12). The CMM, which includes the nuclei raphe pallidus and raphe obscurus, inhibits sympathetic activity through the RVLM, CVLM, and a direct projection to preganglionic sympathetic neurons in the intermediolateral cell column (10, 19, 25, 35, 42, 44). Although less thoroughly investigated than the CVLM, the CMM evidently does not participate in the baroreceptor reflex because inhibition of neuronal activity in the CMM with GABA receptor agonists (10), lidocaine, or cobalt chloride (17) does not affect resting arterial pressure or heart rate. Activation of CMM neurons with excitatory amino acids causes a prompt depressor and sympathoinhibitory response with little or no change in heart rate (10, 11, 16) and overrides the sympathoexcitation produced by baroreceptor activation (11). These observations are consistent with the suggestion that the CMM may mediate the compensatory phase of the response to hemorrhage (17). Hemorrhagic hypotension is inhibited by chemical inactivation of either the CMM or CVLM, however, which suggests that both depressor areas may be involved (17).

The ascending pathways that activate vlPAG neurons in response to hemorrhage remain to be identified. The compensatory phase of hemorrhage is thought to be initiated by cardiopulmonary mechanoreceptors on vagal nerve afferents that synapse in the nucleus of the solitary tract (NTS) (20, 29, 37). The vlPAG receives a direct projection from the NTS (3, 18), which raises the possibility that hemorrhage may activate vlPAG neurons through a short-loop pathway from the NTS. The vlPAG is also influenced by forebrain depressor sites in the amygdala (34), hypothalamus (40), and cortex (15), suggesting, alternatively, that hemorrhage activates the vlPAG through a longer anatomic pathway that involves the forebrain. This latter possibility is supported by evidence that high mesencephalic transection inhibits the compensatory phase of hemorrhage in decerebrate unanesthetized rabbits (14).

Perspectives

It has long been known that severe hemorrhage lowers arterial pressure through a central mechanism, but the anatomic pathway that mediates this response has never been fully elucidated. The present finding that chemical inactivation of the vlPAG inhibits the compensatory phase of hemorrhage provides evidence that the vlPAG is an important component of this pathway. This finding is a logical extension of anatomic evidence that vlPAG neurons innervate two depressor sites in the caudal midline and ventrolateral medulla (8, 10, 16) and that chemical inactivation of either site inhibits hemorrhagic hypotension (17). The compensatory phase of hemorrhage thus appears to be mediated by a descending pathway from the vlPAG to the caudal medulla and the spinal cord. The involvement of the vlPAG in the response to hemorrhage is also a logical extension of physiological data. Activation of vlPAG neurons causes profound opioid-dependent analgesia, for example, and the vlPAG is known to serve a pivotal role in the descending pain control pathway. Recently, we found that opioid receptor antagonists inhibit hemorrhagic hypotension after vlPAG injection (7), which suggests that pain perception and cardiovascular function are controlled by parallel descending pathways during severe injury. Activation of vlPAG neurons also causes behavioral inactivity and hyporeactivity, a syndrome much like that observed during hemorrhage (1). The vlPAG thus appears to be an integrative center that responds to hemorrhage, severe pain, trauma, and inescapable stress to produce a behavioral, antinociceptive, and autonomic repertoire that promotes survival, wound healing, and passive protection from predator attack (1).

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REFERENCES


