5-HT\textsubscript{1A} receptor agonist 8-OH-DPAT acts in the hindbrain to reverse the sympatholytic response to severe hemorrhage

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Serogin, Karie E. 5-HT\textsubscript{1A} receptor agonist 8-OH-DPAT acts in the hindbrain to reverse the sympatholytic response to severe hemorrhage. Am J Physiol Regul Integr Comp Physiol 284:R782–R791, 2003. First published November 7, 2002; 10.1152/ajpregu.00478.2002.—Central administration of serotonergic 5-HT\textsubscript{1A} receptor agonists delays the reflex sympatholytic response to severe hemorrhage in conscious rats. To determine the region where 5-HT\textsubscript{1A} receptor agonists act to mediate this response, recovery of mean arterial pressure (MAP), heart rate (HR), and renal sympathetic nerve activity (RSNA) was compared in hemorrhaged rats after injection of the selective 5-HT\textsubscript{1A} agonist, (+)-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), in various regions of the cerebroventricular system or the systemic circulation. Three minutes after injection of 8-OH-DPAT (48 nmol/kg), MAP and RSNA were higher in hemorrhaged rats given drug in the fourth ventricle (94 ± 5 mmHg, 82 ± 18% of baseline) or the systemic circulation (90 ± 4 mmHg, 113 ± 15% of baseline) than in rats given drug in the Aqueduct of Sylvius (63 ± 4 mmHg, 27 ± 11% of baseline), the lateral ventricle (42 ± 3 mmHg, −8 ± 18% of baseline), or in rats given saline in various brain regions (47 ± 5 mmHg, −42 ± 10% of baseline). A lower-dose injection of 8-OH-DPAT (10 nmol/kg) also accelerated the recovery of MAP and RSNA in hemorrhaged rats when given in the fourth ventricle (94 ± 26 mmHg, 72 ± 33% of baseline 3 min after injection) but not the systemic circulation (46 ± 4 mmHg, −25 ± 30% of baseline). These data indicate that 8-OH-DPAT acts on receptors in the hindbrain to reverse the sympatholytic response to hemorrhage in conscious rats.

(+)-8-hydroxy-2-(di-n-propylamino)tetralin; 5-hydroxytryptamine type 1A

PROGRESSIVE BLOOD LOSS elicits a biphasic cardiovascular response that includes an initial normotensive phase in which a compensatory increase in sympathetic activity helps to maintain blood pressure, followed by a rapid fall in blood pressure, heart rate (HR), and sympathetic activity (46, 48). The mechanism that mediates this reflex sympatholytic response is not known. A better understanding of the central nervous system mechanism that mediates this and related sympatholytic reflex responses, such as vasovagal syncope, exertional syncope with aortic stenosis, and the brady-cardia seen with myocardial infarction of the inferoposterior wall of the ventricle, could lead to more effective treatment of such autonomic dysfunction (36).

Administration of serotonin 5-HT\textsubscript{1A} receptor ligands before either blood loss or simulated hypovolemia by caval vein occlusion has been shown to substantially delay the secondary hypotensive phase in rats and rabbits (19, 20, 48). These early studies led to speculation that endogenous serotonin release mediated the secondary sympatholytic phase of hemorrhage. Of the serotonergic receptor ligands previously tested, the nonselective 5-HT-receptor antagonist methysergide has since been shown to delay the hypotensive response to hemorrhage by an agonist action on 5-HT\textsubscript{1A} receptors (48). Methysergide prevented the hypotensive response into caval vein occlusion when administered in the lateral cerebroventricles, the midbrain pons, or the hindbrain but not the midthoracic intrathecal space of conscious rabbits (20). Although methysergide administration to the lateral ventricle was found to be 10 times more potent in preventing the hypotensive response to hypovolemia than systemic administration, the drug did not differ in its ability to delay hypotension when injected into various brain sites.

The inability to discern a difference in the potency of 5-HT\textsubscript{1A} receptor agonists to delay the depressor response to hemorrhage when given via different central routes of administration is likely because of the relatively large variability in cardiovascular responses that conscious animals exhibit when exposed to blood withdrawal. Moreover, because methysergide is a non-selective serotonergic receptor ligand with some affinity for nonserotonergic receptors, it is possible that other receptor populations could have contributed to its effects, thus obscuring specific 5-HT\textsubscript{1A} receptor-mediated responses (29).

In preliminary studies, we found that intravenous administration of the selective 5-HT\textsubscript{1A}-receptor agonist (+)-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) rapidly reversed the hypotensive and bradycardic responses established during severe hemorrhage with relatively little variability. Consequently, it was reasoned...
that an assessment of the response latency to a more selective 5-HT1A receptor agonist administered to different brain regions of hemorrhaged animals could be used to localize the site of drug action. The regions selected for study in these experiments included 1) the forebrain (via the lateral ventricle), where 5-HT1A receptor agonists have been shown to promote the release of circulating peptides that influence sympathetic activity (5, 44); 2) the Aqueduct of Sylvius that lies directly above the ventro-lateral periaqueductal gray (vPAG), a region in which opioid receptor blockade prevents the depressor response to hemorrhage (13); and 3) the medulla (via the 4th ventricle) where both somatodendritic 5-HT1A autoreceptors and postsynaptic 5-HT1A receptor targets of serotonergic neurons are expressed within nuclei that regulate cardiovascular and respiratory function (25, 26, 53, 54). Finally, responses to systemic administration were also studied to assess a possible peripheral site of action. It was reasoned that injections of 5-HT1A receptor agonist made closest to the site of action would produce the most rapid recovery of blood pressure, HR, and sympathetic activity.

METHODS

Animals

Male Sprague-Dawley rats weighing between 350 and 400 g (Harlan, Indianapolis, ID) were housed individually and given ad libitum access to food and water for at least 1 wk before surgery. The housing facility was maintained at a constant temperature of 22 ± 2°C with a light-dark cycle of 12:12 h. All experiments were conducted in accordance with the American Physiological Society’s Guiding Principles for Research Involving Animals and Human Beings (1) and were approved by the University Institutional Animal Care and Use Committee.

Surgery

Cannulations. At least 10 days before the experiment, rats were randomly assigned to four groups, three of which were fitted with a guide cannula in one of three sites within the cerebral ventricular system to enable microinjection of drug to various regions. After pentobarbital sodium anesthesia (60 mg/kg ip), stereotaxic surgical procedures were employed to implant guide cannulas, made from 25-gauge hypodermic tubing, in either the right lateral ventricle, the Aqueduct of Sylvius, or the fourth ventricle dorsal to the caudal pole of the facial motor nucleus. The cannulas were placed according to the coordinates of Paxinos and Watson, i.e., lateral ventricle: 1.6 mm lateral and −1 mm posterior to bregma and −2.6 mm ventral from the dura mater; Aqueduct: +0.7 mm to the interaural line at the midline and −5.3 mm ventral from the skull surface; and fourth ventricle: −2.6 mm relative to the interaural line at the midline and −7.4 mm ventral from the skull surface (45). All cannulas were cemented with dental acrylic to jeweler’s screws secured to the skull through burr holes. The rats were returned to the animal quarters after recovery from anesthesia.

Electrode and vascular catheters. Electrode and vascular catheters were implanted as described previously (49). Briefly, 24 h before the experiment, the rats were anesthetized (60 mg/kg ip pentobarbital sodium) and implanted with bilateral femoral arterial catheters and a unilateral femoral venous catheter (PE-50 heat welded to a length of PE-10) to enable direct measurement of mean arterial pressure (MAP), arterial blood withdrawal, and drug injection. During the same surgery, a stainless steel, Teflon-coated (bare diameter = 0.005 in.; A-M Systems, Everett, WA) bipolar renal nerve recording electrode was implanted through a left flank incision. First, the electrode connector was externalized subcutaneously along with the vascular catheters at the nape of the neck. The left kidney was then retracted, and a multifiber nerve bundle was isolated as it projected to the kidney parallel with the renal artery. The nerve was placed on the electrode leads and embedded in a lightweight dental silicon (Bisco S4i; Bisco, Bielefeld, Germany). The flank incision was suture closed in two layers with the electrode leads coiled within the subcutaneous space. The rats were allowed to recover overnight in their home cage.

Data Acquisition

During all experiments, arterial pressure, HR, and renal sympathetic nerve activity (RSNA) were recorded continuously on a Macintosh G3 laptop computer using PowerLab data acquisition software (Chart version 3.6.1; ADInstruments, Grand Junction, CO). The arterial pressure was measured with a disposable pressure transducer (Abbott Laboratories, North Chicago, IL) and a bridge amplifier (ADInstruments). HR was calculated on-line using peak-to-peak detection of the pulse pressure wave. Sympathetic activity was sampled (4,000 Hz), amplified (10–20,000 times), and filtered (1–3,900 Hz) with a PowerLab Bioamplifier (ADInstruments). The recorded neurogram was integrated off line by calculating the root mean square (RMS) over 20-ms time bins. Background noise in the nerve recording was determined at the end of the experiment after elimination of efferent nerve activity 2 min after injection of the ganglionic blocker hexamethonium chloride (30 mg/kg iv). Background noise was subtracted from averaged nerve activity to provide a measurement of RSNA. All measurements of RSNA were normalized (%Δbaseline) to basal nerve activity determined over a 10-min period directly before hemorrhage. Only data from visibly healthy animals with greater than a 2.1 signal-to-noise ratio in their nerve recording signal were included in the study.

Experimental Design

Before the experiment, the animals were connected to the recording instrumentation and a withdrawal pump while resting unrestrained in their home cage. The injector containing drug for brain injection was filled with appropriate fluid and placed in the guide cannula before habituation. The rat was then allowed to rest undisturbed for at least 2 h before hemorrhage. Arterial pressure, HR, and RSNA were recorded continuously beginning 10 min before the hemorrhage and ending 20 min after hemorrhage termination. Controlled blood withdrawal was initiated at a rate of 3.2 ml·min⁻¹·kg⁻¹ for 6 min, after which the speed was reduced to 0.55 ml/min for an additional 4 min. In preliminary tests, this procedure was found to produce a consistent fall in MAP, HR, and RSNA after withdrawal of ~11.2 ml/kg blood or ~14% of estimated blood volume. The subsequent change to the lower rate of withdrawal was sufficient to maintain bradycardic and sympatholytic responses until hemorrhage termination.

After the initiation of blood withdrawal (7 min), 48 nmol/kg 8-OH-DPAT (in 0.5 μl saline) or an equivalent volume of saline was injected over 20 s in either the lateral ventricle, the Aqueduct of Sylvius, or the fourth ventricle. In a fourth group, drug or vehicle was injected intravenously in
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a volume of 5 μl and flushed with an additional 100 μl of saline over 20 s. All injections were made remotely via tubing that extended outside the cage. After drug injection, no further intervention or resuscitation was performed before termination of the experiment. The dose of 8-OH-DPAT chosen for use in these initial studies corresponded to the ED₅₀ determined in prior experiments to increase the volume of blood withdrawal that produced a 40-mmHg fall in blood pressure when injected in the lateral ventricle 15 min before hemorrhage (48). In a separate set of experiments, responses to a lower dose (10 nmol/kg) injection of 8-OH-DPAT in the fourth ventricle or systemic was assessed after hemorrhage in the same manner. An additional sham-hemorrhage group, instrumented in the same manner, was injected with 10 nmol/kg 8-OH-DPAT in the fourth ventricle without prior hemorrhage.

After termination of the experiment, rats were killed with an overdose of pentobarbital sodium (100 mg/kg iv). Cannulated rats were subsequently given intercerebroventricular injections of 0.5 μl toluidine blue (0.5%). The brains were removed quickly and postfixed in 10% formalin overnight. The brains were cut the following day to confirm proper cannula placement. Only data from animals in which dye was found to have diffused through the ventricular system (i.e., dye was not injected in tissue) were included in the data analysis.

Data Analysis

Infusion of saline resulted in the same cardiovascular responses to hemorrhage, regardless of the site of infusion. Therefore, data from all saline-treated rats, irrespective of the site of injection, were pooled for comparison with drug-injected groups. Control data used for comparison with groups given 48 nmol/kg 8-OH-DPAT were comprised of rats given saline in the lateral ventricle (n = 1), the Aqueduct (n = 1), the fourth ventricle (n = 2), and intravenously (n = 2).

Data were averaged into 1-min blocks for analysis. Two-way ANOVAs with repeated measures were used to determine group differences in response to blood withdrawal during the initial 7 min of hemorrhage before drug injection. Additional two-way ANOVAs were used to compare effects of route of drug administration in hemorrhaged rats over time at 5-min intervals between the onset of hemorrhage until termination of the recording period (20 min after hemorrhage termination). Significant interactions between treatment and time were followed up with a post hoc Tukey/Kramer’s test to compare group means at each time point. P values <0.05 were accepted as significant.

RESULTS

Repeated-measures ANOVA of BP, HR, and RSNA responses beginning from the initiation of blood withdrawal through the succeeding 7 min (i.e., before drug injection) indicated that responses to blood withdrawal per se did not differ between groups. Therefore, group data obtained before drug administration were pooled to characterize changes in MAP, HR, and RSNA during the first 7 min of hemorrhage (Fig. 1). Blood withdrawal caused an initial rise in sympathetic activity, whereas MAP and HR were maintained. As noted in Fig. 1, RSNA continued to rise after the third minute of blood withdrawal, by which time blood pressure had fallen significantly. The fall in HR only became significant after the 5th min of blood withdrawal.

After saline administration, 7 min after initiation of hemorrhage, blood pressure began to rise slowly (see control data, Fig. 2). After hemorrhage termination, blood pressure continued to recover slowly, reaching a subbaseline plateau ~12 min after hemorrhage termination. HR and RSNA remained suppressed throughout hemorrhage. Both HR and RSNA recovered more slowly than did blood pressure. Bradycardia was maintained throughout the posthemorrhage measurement period. RSNA also recovered more slowly than did blood pressure. However, basal activity was reestablished within 20 min of hemorrhage termination.

8-OH-DPAT (48 nmol/kg) injection given after establishment of hypotension significantly accelerated recovery of all parameters (Fig. 2). However, the effect differed with route of administration (P < 0.001). Systemic and fourth ventricle 8-OH-DPAT administration produced the most rapid recovery of blood pressure, as indicated by the significantly higher pressures observed in the two aforementioned groups compared with all other groups 3 min after injection (P < 0.01). Injection of drug in the Aqueduct produced an intermediate response characterized by a slight acceleration of blood pressure recovery, resulting in pressure levels that were significantly greater than lateral ventricle-injected rats, but not control rats, 3 min after drug administration. Eight minutes after injection (15 min after initiation of hemorrhage), blood pressure in all drug-treated groups had reached a plateau near baseline levels. At this point, the blood pressure of saline-treated rats continued to rise but remained signifi-
cantly lower than all 8-OH-DPAT-treated groups ($P < 0.01$).

HR showed a biphasic response after fourth ventricle and systemic injection of 8-OH-DPAT. In these groups, HR recovered rapidly during the first 3 min after injection. However, recovery abruptly stopped and appeared to decline to a plateau well below baseline. Both systemic- and fourth ventricle-treated rats had higher HR than control or lateral ventricle-injected rats 3 min after injection. Rats injected with drug in the Aqueduct showed an intermediate response with no differences between any of the groups 3 min after injection.

Rats treated with systemic and fourth ventricle 8-OH-DPAT also showed a rapid rise in RSNA such that values were greater than either saline or lateral ventricle-injected rats 3 min after injection. Aqueduct injection produced an intermediate sympathetic response, such that RSNA slowly rose to levels significantly higher than that of control rats 8 min after injection. 8-OH-DPAT produced a slower acceleration of RSNA recovery when injected in the lateral ventricle, with significantly higher activity achieved 8 min after injection.

Systemic and fourth ventricle injection of 8-OH-DPAT (48 nmol/kg) produced parallel cardiovascular responses when administered after hypotensive hemorrhage. To rule out the possibility that the effects of fourth ventricle injection of 8-OH-DPAT were because of diffusion of drug in the peripheral circulation, additional experiments were performed in which responses to a fivefold lower dose of drug administered in the fourth ventricle or systemic circulation were compared.

Responses to a 10 nmol/kg injection of 8-OH-DPAT in the fourth ventricle also produced a rapid reversal of hypotension, bradycardia, and sympathoinhibition in hemorrhaged rats (Fig. 3). In contrast, the same dose had little effect on recovery when given systemically (Fig. 4). Indeed, comparisons of MAP, HR, and RSNA indicated a highly significant difference between the two groups 3 min after injection. Although HR responses began to plateau shortly after fourth ventricular injection of drug, differences in MAP and RSNA between the two groups persisted even 18 min after injection. Sham-hemorrhaged rats treated with the lower dose of 8-OH-DPAT in the fourth ventricle showed no cardiovascular or sympathetic responses to drug administration (Table 1).

Injection of 0.5 µl toluidine blue dye in the right lateral ventricle diffused well through the forebrain ventricular system, as evidenced by obvious dye penetration in the contralateral lateral ventricle (Fig. 5A). However, there was only limited evidence of dye in the fourth ventricle at the rostral-caudal level of the caudal facial motor nucleus. Successful injection of dye in the Aqueduct resulted in dye penetration well into the surrounding periaquaductal regions and the underlying dorsal raphe nucleus (Fig. 5B). Aqueduct injection produced light staining within the fourth ventricle, with only limited diffusion to more rostral sites along the third ventricle. Successful injections in the fourth ventricle showed extensive dye penetration through
the dorsal surface of the hindbrain with evidence of limited diffusion around the lateral sides of the brainstem (Fig. 5C). Fourth-ventricle injection of dye diffused to a limited extent in the rostral region of the Aqueduct.

**DISCUSSION**

In a previous study, we demonstrated that serotonergic agonists act on central nervous system 5-HT$_{1A}$ receptors to delay the onset of the hypotension, bradycardia, and sympathoinhibition that normally result from severe blood loss (48). In the present study, the region where 5-HT$_{1A}$ receptor agonists act to influence the hypertensive and sympatholytic responses to hemorrhage was determined by comparing recovery of MAP, HR, and RSNA immediately after injection of the selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT in different brain regions and the peripheral circulation after severe hemorrhage. Recovery of MAP, HR, and RSNA was most rapid after fourth ventricle and systemic injection of 8-OH-DPAT. Aqueduct injection produced an intermediate response, whereas lateral ventricle injection produced the slowest onset of effect. A substantially smaller dose of drug given in the fourth ventricle continued to accelerate recovery of MAP and RSNA to the same extent. However, the same low dose of 8-OH-DPAT given systemically no longer accelerated recovery compared with saline-treated controls. Together the data support the view that the activation of 5-HT$_{1A}$ receptors in the hindbrain is responsible for the accelerated recovery from hypertensive hemorrhage mediated by 8-OH-DPAT.

The hindbrain houses the major central nervous system sites involved in the reflex regulation of cardiovascular homeostasis. For instance, the decrease in pulse pressure that follows blood loss reduces the activity of arterial baroreceptor afferents (baroreceptor unloading) that terminate within the nucleus of the solitary tract (NTS; see Ref. 24) in the dorso medial surface of the caudal medulla near the obex. During increases in pressure, excitatory projections from the NTS normally activate inhibitory GABAergic interneurons of the caudal ventrolateral medulla (cVLM). The cVLM, in turn, provides inhibitory input to the adjacent rostral portion of the ventrolateral medulla (rVLM) that regulates tonic descending excitatory drive to preganglionic sympathetic neurons of the spinal cord. Sensory afferent activity from both arterial and cardiopulmonary baroreceptors is decreased during mild hemorrhage (56). Thus it can be inferred that reduced activity of baroreceptor afferents results in disinhibition of the bulbospinal excitatory projections to the preganglionic sympathetic nerves of the spinal cord during hemorrhage. Rats, rabbits, and dogs subjected to arterial baroreceptor denervation show no compensatory response to blood loss, suggesting that the maintenance of blood pressure during mild hemorrhage is heavily dependent on the same central nervous system pathways associated with baroreflex control of blood pressure (15, 31, 40, 52, 55).

Results from the present study are intriguing in that HR and RSNA did not appear to decline significantly until after blood pressure began to fall. These data bring into question the view that loss of sympathetic-mediated peripheral resistance per se mediates the fall in blood pressure during severe hemorrhage. Alternatively, it is likely that blood pressure began to fall only after the compensatory responses began to plateau.
Presumably, the relatively weak compensatory responses that occur during initiation of the sympatholytic phase may not have been able to fully offset the decline in cardiac output.

Very little is known about the afferent mechanisms that mediate the sympatholytic response to excessive blood loss. Studies suggest that a class of cardiac mechanoreceptors distinct from those unloaded by mild hemorrhage may be responsible for the sudden reflex sympathoinhibition characteristic of more severe hemorrhage (42, 43). Although this view remains controversial, it has been speculated that a sympathetic-mediated increase in the vigor of ventricular contraction during the initial stage of hemorrhage eventually contributes to a paradoxical activation of mechanoreceptor afferents when ventricular filling becomes extremely low. Presumably, ventricular contraction against little or no blood volume activates mechanoreceptors that initiate immediate and profound bradycardia and sympathoinhibition (42, 43). The central nervous system pathway involved in the sympathoinhibitory response to mechanosensitive afferent activation has only been studied to a limited extent. Direct stimulation of cardiac mechanosensitive fibers activates NTS neurons (50). However, it is not clear whether these are the same neurons that mediate reflex activation of rVLM GABAergic interneurons of the baroreflex pathway. Interestingly, rats subjected to chronic NTS lesion show a tachycardic, rather than a bradycardic, response to severe hemorrhage, suggesting that NTS activation may indeed play a role in the sympatholytic response to hemorrhage in rats (47).

Anatomic studies have provided additional, albeit indirect, evidence that the baroreflex pathway is involved in mediating the neural response to hemorrhage. Blood loss induces expression of protein markers of cellular activation (i.e., the immediate-early gene product, Fos) in nuclei involved in the baroreflex pathway, including the NTS, the rVLM, and the cVLM (14). Additionally, the lateral parabrachial nucleus (LPBN), the locus coeruleus, the vlPAG region as well as the superoptic nucleus (SON), and both magnocellular and parvocellular regions of the paraventricular nucleus (PVN) also show Fos expression in response to hypotensive hemorrhage (32, 33). The precise pathway that leads to activation of forebrain sites during hemorrhage is not clear but may be related, in part, to adrenergic stimulation derived from the locus coeruleus, the NTS, or the A1 cells of the cVLM region. In general, the same nuclei show Fos expression in response to both mild and severe hemorrhage (3, 4). As yet, Fos expression studies have not selectively identified neuronal populations involved specifically in the sympatholytic phase of hemorrhage. Of these sites that express Fos in response to hemorrhage, the NTS, rVLM, cVLM, vlPAG, PVN, and SON have been shown to express 5-HT_{1A} receptors (53, 54, 59, 60). However, the functional response to 5-HT_{1A} receptor stimulation in many of these regions has not yet been characterized. Nonetheless, the ability of 8-OH-DPAT to reverse the sympatholytic effect of severe hemorrhage could be due to a direct effect of the drug on neuronal function within these nuclei.

5-HT_{1A} receptor stimulation in the PVN causes the release of peptides that influence cardiovascular regulation, including oxytocin and corticotropin-releasing hormone (5, 44). However, it is unlikely that increases in circulating hormones mediated the pressor response...
to 8-OH-DPAT during hemorrhage. First, lateral ventricle injection of 8-OH-DPAT would have distributed rapidly to forebrain 5-HT_{1A} receptors that initiate oxytocin and corticotropin-releasing factor release. Yet lateral ventricle injection elicited much slowerpressor and sympathoexcitatory responses than did hindbrain injection of 8-OH-DPAT. Second, the pressor and sympathoexcitatory responses that occurred during hindbrain injection of 8-OH-DPAT would appear to be far too rapid (<30 s) to be mediated by hormone release. Indeed, 5-HT_{1A} receptor-mediated increases in plasma corticosterone levels take several minutes to develop (6). Furthermore, prior studies indicate that lesion of the parvocellular PVN sufficient to reduce hemorrhage-induced corticosterone release has little influence on blood pressure and HR responses to hemorrhage (9). Although oxytocin release could conceivably mediate more rapid effects, there is currently little evidence that oxytocin modulates sympathetic-mediated regulation of blood pressure (28, 58).

Recent work indicates that the hypotensive response to severe blood loss is mediated, in part, by neurons in the vPAG region. Specifically, bilateral blockade of synaptic transmission in the vPAG was shown to delay and attenuate the extent of the depressor response to hemorrhage in conscious rats (12). In a subsequent study, bilateral δ-opioid receptor blockade in the vPAG was found to prevent the depressor response to blood withdrawal (13). Given the high density of 5-HT_{1A} receptors expressed in this region, the possibility that 5-HT_{1A} agonists might act within the vPAG to reverse the hypotensive and sympatholytic responses to hemorrhage was assessed by injecting drug in the Aqueduct just dorsal to this region. It was found that dye injected in successfully placed Aqueduct cannulas permeated the vPAG region. However, MAP, HR, and RSNA rose significantly more slowly after Aqueduct injection of 8-OH-DPAT than after fourth ventricle injection of drug. The data indicate that 8-OH-DPAT likely did not reverse the hypotensive response to hemorrhage by an action on neurons of the vPAG. The slower action of Aqueduct vs. fourth ventricle injection further indicates that 8-OH-DPAT probably does not act by stimulating somatodendritic autoinhibitory 5-HT_{1A} receptors in the dorsal or median raphe to limit serotonin release at their respective projection sites.

Lesion of the LPBN also influences cardiovascular responses to hemorrhage. Specifically, full lesion of the nucleus delays the onset of blood pressure recovery after hemorrhage, whereas partial lesion actually accelerates recovery (8). In our studies, 8-OH-DPAT was administered in the fourth ventricle ~2 mm caudal to the parabrachial complex. Although it is conceivable that the drug diffused rostrally to the region, it is unlikely that the drug mediated its effects in the LPBN since administration of 8-OH-DPAT before hemorrhage was previously shown to delay the onset of hypotension. In contrast, neither partial nor full lesion of the LPBN has any effect on the onset of the sympatholytic phase of hemorrhage.

Evidence that hindbrain injection of a selective 5-HT_{1A} agonist rapidly reversed the hypotensive and sympatholytic responses to hemorrhage confirms findings by Evans et al. (19), indicating that intracisternal injection of the nonselective serotonergic receptor ligand methysergide delays the hypotensive response to central hypovolemia in conscious rabbits (19, 20). We have since shown that methysergide delays the hypotensive response to hemorrhage in conscious rats by an agonist action on 5-HT_{1A} receptors (48). Thus it seems likely that methysergide and 8-OH-DPAT act at the same site to mediate their respective effects during hemorrhage in both rat and rabbit. However, the mechanism of action remains obscure.

It is possible that the rapid response to hindbrain injection of 8-OH-DPAT was the result of diffusion of the drug to more caudal sites within the spinal cord. Difficulty encountered in performing intrathecal injections just caudal to the fourth ventricle in conscious rats precluded our ability to test this possibility directly. However, Evans et al. (19) previously reported no discernable effect of methysergide on hemorrhage responses after injection in the thoracic intrathecal space (T_{8}) of conscious rabbits (20). Moreover, it is likely that the time required for drug to diffuse from the fourth ventricle to spinal cord segments that send preganglionic neurons to the kidney (T_{8}–T_{10}) would have been longer than the latency to response seen during hemorrhage.

Table 1. Response to 10 nmol/kg 8-OH-DPAT in euvoletic rats

<table>
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<th>Time, min</th>
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<td>MAP, mmHg</td>
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<td>12 ± 10</td>
<td>8 ± 12</td>
<td>−17 ± 15</td>
<td>5 ± 30</td>
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Values are means ± SE of mean arterial pressure (MAP), heart rate (HR), and renal sympathetic activity (RSNA) measured at increasing time intervals after 10 nmol/kg injection of (+)-(S)-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in the 4th ventricle of sham-hemorrhaged rats (n = 4). Repeated-measures ANOVAs did not reveal any significant effect of drug injection.

Fig. 5. Typical examples of diffusion pattern of toluidine blue dye (0.5 μl) injected in properly placed right lateral ventricle (A), Aqueduct of Sylvius (B), and the fourth ventricular (C) cannulas.
after fourth ventricle injection. Also, both the tachycardic and sympathoexcitatory responses to fourth ventricle drug injection occurred at the same time, suggesting that reflex responses in higher-order neurons likely mediated the response.

The fourth ventricle and systemic administration of 8-OH-DPAT (48 nmol/kg) produced a similar rapid recovery of blood pressure and sympathetic activity. The more rapid and potent recovery from hemorrhage after systemic administration of the higher dose of 8-OH-DPAT compared with that after forebrain or Aqueduct injection was likely the result of a more rapid distribution of drug to the medulla via the vascular circulation than through the sluggish ventricular system. Indeed, radioactive tracer studies have shown that [14C]sucrose injected in the lateral ventricle of the rat reaches its peak concentration within the cisterna magna of the hindbrain >10 min after injection, further demonstrating the relatively slow diffusion through the ventricular system (22, 23).

8-OH-DPAT is relatively lipophilic and readily crosses the blood-brain barrier (34). Consequently, it is possible that drug administered centrally diffused to the circulation to act on a peripheral site of action. Although such peripheral diffusion is not unprecedented, a peripheral effect of centrally administered drug would presumably have had a similar latency of effect after injection in different central sites. Moreover, a possible systemic action after fourth ventricular injection seems unlikely given that a fivefold lower dose of 8-OH-DPAT was no longer effective when given systemically but was just as effective at reversing the hypotensive and sympatholytic response to hemorrhage when given in the fourth ventricle as when given systemically at the higher dose. Certainly, these data do not permit any precise conclusion as to the location of the medullary 5-HT1A receptor population on which 8-OH-DPAT acts to mediate its effects. However, the rapidity of the response and the apparent lack of diffusion of dye to the ventral surface of the medulla would suggest that receptors in the dorsal region were most likely responsible for the sympathoexcitatory effect of the drug. In accordance, immunohistochemical and receptor binding studies have demonstrated a high density of 5-HT1A receptors within the dorsal medullary region, including the area postrema and particularly the NTS (25, 53). Although it is possible that fourth-ventricle injection of drug diffused around the hindbrain to the ventral surface to influence the rVLM, this seems less likely given the well-documented sympathoinhibitory effect of 8-OH-DPAT on rVLM neurons in anesthetized rats (41). The lack of any sympathoinhibitory effect of 8-OH-DPAT when given in the fourth ventricle of euvoletic rats in the present study suggests that the drug did not reach the ventral surface of the hindbrain.

It is not known how 5-HT1A receptor stimulation reversed hypotensive hemorrhage. It is possible that activation of serotonergic somatodendritic autoreceptors in the hindbrain may have reversed the hypotensive response to hemorrhage by reducing endogenous serotonin release. A role for endogenous serotonin release in the sympatholytic response to hemorrhage has been supported in the literature. Earlier work by Elam et al. (18) indicated that serotonin depletion prevented the hypotensive response to hemorrhage in anesthetized cats. Similar results were also observed in rats (39). WAY-100635 administered directly in rVLM was shown to prevent the sympatholytic response to hemorrhage in pentobarbital sodium-anesthetized rats (17). However, partial depletion of central serotonin with either repeated applications of p-chlorophenylalanine or central injection of the selective serotoninergic neurotoxin 5,7-dihydroxytryptamine failed to influence the hypotensive response to caval vein occlusion in conscious rabbits (20). We also showed that systemic administration of the highly selective 5-HT1A-receptor antagonist WAY-100635 failed to influence the hypotensive response to hemorrhage in conscious rats (48). Moreover, activation of 5-HT1B receptors, which act as serotonergic nerve terminal autoreceptors, also failed to delay the hypotensive response to hemorrhage in the conscious rat, suggesting that suppression of serotonin release does not mediate the ability of 8-OH-DPAT to reverse the hypotensive response to hemorrhage (48).

It remains to be determined why our more global blockade of 5-HT1A receptors failed to delay the hypotensive response to hemorrhage. It is possible that blockade of other 5-HT1A receptor populations confounded the effects of rVLM 5-HT1A receptor blockade. Alternatively, the discrepancy may lie with anesthesia. Anesthesia has profound effects on both responses to hemorrhage and serotonergic neural and receptor function (10, 27, 38, 51, 57). It is tempting to speculate that altered serotonergic function seen during anesthesia may in part mediate the altered hemorrhage responses seen in anesthetized animals.

In conclusion, the data shown here indicate that the 5-HT1A-receptor agonist 8-OH-DPAT acts within the hindbrain to reverse the hypotensive response to hemorrhage in conscious rats. Given that selective 5-HT1A receptor antagonists given in the systemic circulation have been shown to block the ability of both 8-OH-DPAT and methysergide to delay the sympatholytic responses to severe hemorrhage in rats and rabbits, it is likely that these responses are also mediated by 5-HT1A receptors in the hindbrain.

Perspectives

5-HT1A receptors are coupled to the G\textsubscript{i}/G\textsubscript{o}/G\textsubscript{z} family of heterotrimeric G proteins. In response to activation of these different G proteins, 5-HT1A receptor activation mediates various cellular responses, including increased potassium conductance, decreased adenyllyl cyclase activity (and consequently decreased cAMP levels), and decreased calcium currents (2, 7, 11, 21). In all cases, 5-HT1A receptor activation is associated with neuronal hyperpolarization. As such, the responses to 5-HT1A receptor stimulation observed in hemorrhaged animals likely resulted from an acute disinhibition of the sympathetic nervous system. This response to drug
appears to occur selectively during sympathoinhibition, since drug administration in euvoletic rats has little effect. In fact, other studies indicate that central 5-HT_{1A} agonist administration in euvoletic anesthetized animals causes a substantial decrease in sympathetic activity (16, 30). Microinjection studies suggest that this sympatholytic response likely results from direct inhibition of descending preautonomic sympathetic neurons in the rostral ventrolateral medulla (35, 37, 41). Indeed, a subset of adrenergic C_{1} neurons that comprise ~50% of the descending bulbo-spinal presynaptic sympathetic neural population expresses 5-HT_{1A} receptors (25). The most parsimonious explanation for this divergence of effects of 5-HT_{1A} receptor activation in euvoletic and hypovolemic animals is that severe hypovolemia activates some as yet unidentified sympathoinhibitory pathway that is sensitive to 5-HT_{1A} receptor-mediated inhibition. As such, identification of the precise locus where 5-HT_{1A} receptor agonists act to reverse the hypotensive response to hemorrhage may lead to a better understanding of the trigger and/or central nervous system pathways that contribute to the rapid loss of blood pressure during severe hemorrhage, neither of which has been delineated to date.

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