Methysergide delays the decompensatory responses to severe hemorrhage by activating 5-HT$_{1A}$ receptors

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Scrogin, Karie E., Alan Kim Johnson, and Virginia L. Brooks. Methysergide delays the decompensatory responses to severe hemorrhage by activating 5-HT$_{1A}$ receptors. Am J Physiol Regulatory Integrative Comp Physiol 279: R1776–R1786, 2000.—Central administration of the serotonin receptor ligand methysergide delays the decompensatory response to hypotensive hemorrhage. This study was performed to determine the receptor subtype that mediates this effect. Lateral ventricular (LV) injection of methysergide (40 μg) delayed the hypotensive, bradycardic, and sympathoinhibitory responses to blood withdrawal (1.26 ml/min) in conscious rats. The response was quantified, in part, as the blood volume withdrawal that produced a 40-mmHg fall in blood pressure. The delayed hypotensive response produced by methysergide (8.2 ± 0.2 vs. 5.6 ± 0.2 ml, P < 0.01) was reversed by the 5-hydroxytryptamine (HT)$_{1A}$ antagonist WAY-100635 (30 μg iv: 6.7 ± 0.4 ml, P < 0.01; 100 μg iv: 5.6 ± 0.1 ml, P < 0.01). LV injection of the 5-HT$_{1A}$ agonist (+)-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) also delayed the hypotensive (10 μg: 8.6 ± 0.3, P < 0.01; 20 μg: 9.2 ± 0.3 ml, P < 0.01), bradycardic, and sympathoinhibitory responses to hemorrhage. WAY-100635 (10 μg iv) completely reversed the effects of 8-OH-DPAT (20 μg: 5.4 ± 0.3 ml). Neither selective blockade of 5-HT$_{2}$ receptors nor stimulation of 5-HT$_{1B}$, 5-HT$_{1D}$ receptors had any effect on hemor-

grophic blood loss stimulates a complex array of neuroendocrine responses that help to maintain blood pressure during the initial fall in blood volume. After a critical blood volume loss (20–30%), however, a second, decompensatory phase ensues that is characterized by a sudden loss of sympathetic drive, reduced vascular resistance, and hypotension (34). The trigger responsible for the sudden failure of the sympathetic compensatory response is unknown.

It has been suggested that central serotonin release may be involved in this secondary decompensatory response. Specifically, serotonin depletion has been shown to attenuate the bradycardic and hypotensive responses to severe blood loss in anesthetized cats (12). The same treatment was shown to abolish the bradycardic and sympathoinhibitory responses observed during hypotensive hemorrhage in anesthetized rats (28). Systemic administration of the nonselective 5-hydroxytryptamine (5-HT)-receptor antagonist methysergide was also shown to accelerate the recovery of blood pressure and heart rate (HR) after severe blood loss in cats and to prevent the bradycardic and sympatholytic responses to hypotensive hemorrhage in anesthetized rats (12, 28). Central administration of methysergide also prevented the fall in blood pressure and peripheral resistance observed in rabbits subjected to caval vein occlusion (15, 16). Our own studies demonstrated that central methysergide administration abolished the bradycardia and attenuated the renal sympathoinhibitory response to hemorrhage in conscious rats at a dose that was ineffective when given systemically (40). Together, these data suggest that endogenous central serotonin release causes or contributes to the decompensatory response to severe hemorrhage.

Nevertheless, the notion that endogenous serotonin release leads to decapsulation during hemorrhage has come into question. In more recent studies, conscious, serotonin-depleted rabbits showed normal biphasic cardiovascular responses to central hypovolemia, suggesting that blockade of hemorrhage-induced bradycardia and sympathoinhibition observed with serotonin depletion in prior studies was due, in part, to a confounding effect of anesthesia (16). Moreover, it is unclear whether a purely antagonist action is responsible for the attenuated decompensatory response to hemorrhage observed after methysergide administration. Though methysergide has potent antagonist activity at a number of serotonergic receptor subtypes, the drug also shows potent agonist actions on 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptors (23). Indeed, Evans et al. (15) speculated that stimulation of 5-HT$_{1A}$ receptors was responsible for the ability of methysergide to prevent hypotension during hypovolemia in the rabbit. The lack of a highly selective, potent antagonist for the 5-HT$_{1A}$ receptor prevented confirmation of this hypoth-

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esis. With the recent development of more potent and selective antagonists, investigation of the pharmacological mechanism by which methysergide acts to alter hemorrhage responses is now feasible. Therefore, this study was performed to determine the receptor subtype responsible for the ability of methysergide to attenuate the decompensatory response to hemorrhage in conscious rats.

METHODS

Animals

All subjects were male Sprague-Dawley rats weighing between 350 and 400 g. Animals were housed individually in wire cages in the Institution’s Animal Care Unit with ad libitum access to Purina rat chow and tap 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 procedures were performed in accordance with the Institution’s guidelines for the care and use of experimental animals.

Surgery

All subjects were anesthetized (pentobarbital sodium, 25 mg ip) and implanted with a right lateral ventricular (LV) cannula (23-gauge hypodermic tubing) at least 10 days before the experiment, as described previously (36). Twenty-four hours before the experiment, the rats were reanesthetized (pentobarbital sodium, 25 mg ip) and implanted with a single femoral venous catheter (Tygon, Norton Performance Plastics, Akron, OH), bilateral femoral arterial catheters (PE-50 heat-welded to a length of PE-10), and a stainless steel, bipolar recording electrode made from single-stranded, Teflon-coated wire (bare diameter 0.005 in., A-M Systems, Everett, WA) soldered to a female microconnector (Microtech, Bovington, PA). The vascular catheters were tunneled subcutaneously to exit at the nape of the neck along with the electrode assembly that was positioned through a left-flank skin incision. The abdominal and back muscles were accessed through the same flank incision, dissected, and retracted to expose the renal artery. A 1- to 2-mm length of sympathetic nerve emanating from the aorticorenal ganglion was isolated and placed on hooks formed at the end of the electrode leads. The nerve and hooks were embedded in a light-weight dental silicon (Bisco, Bielefeld, Germany). The flank incision was sutured closed in two layers with the electrode leads coiled within the subcutaneous space. The rats were allowed to recover in their home cage for 24 h.

Drugs

Pentobarbital sodium was obtained from Abbott Laboratories (North Chicago, IL). Hexamethonium chloride, used to block postganglionic nerve activity, was obtained from Sigma. All other drugs were obtained from Research Biochemicals International (Natick, MA) including the nonselective serotoninergic ligand methysergide maleate, the selective 5-HT1A-receptor antagonist LY-53857 maleate, the 5-HT1A-receptor agonist (+)-8-hydroxy-2-(di-n-propylamino)tetrail (8-OH-DPAT), the selective 5-HT1A-receptor antagonist WAY-100635, and the selective 5-HT1B/D agonist CGS-12066. All drugs were diluted in isotonic saline except methysergide and CGS-12066, which were dissolved in 20% 2-hydroxy-propyl-β-cyclodextran diluted (w/v) in isotonic saline.

Data Acquisition

During the experiment, arterial pressure, HR, and renal sympathetic nerve activity (RSNA) were recorded continuously. Arterial pressure and HR were recorded with a Statham P23 transducer, a Grass bridge preamplifier (7P1), and a Grass tachograph (7P4). Nerve activity was detected with a Grass high-performance AC preamplifier (P511) via a Grass high-impedance probe (F-HIP511G). Raw nerve activity was filtered (high pass: 100 Hz, low pass: 3,000 Hz) and amplified (×20–50,000). The filtered and amplified neural activity was monitored visually with an oscilloscope (model 2212, Tektronix, Beaverton, OR) and simultaneously fed through a Grass integrator (7P10) for full-wave rectification. The resulting voltage signal was integrated over 1-s intervals. The level of background noise in the nerve recording was determined by blocking ganglionic transmission (hexamethonium chloride, 30 mg/kg iv) at the end of each experiment. The noise was subtracted from the entire recording, and the remaining activity was expressed as a percent change from baseline activity determined before any drug treatment (average activity for a 5-min period immediately before the first drug or vehicle injection).

Analog arterial pressure, HR, and integrated RSNA signals from the amplified sources were sampled by computer using an analog-to-digital conversion card (Data Translation, DT2812, Marlboro, MA). The signals were sampled at 200 Hz. The values were collected using LabTech Notebook software (version 7.1.1, Laboratory Technologies, Wilmington, MA).

Experimental Protocols

Experimental protocol 1: assessment of the effect of central methysergide on cardiovascular and sympathetic responses to hemorrhage. The purpose of this protocol was to quantify the degree to which central administration of methysergide attenuates the hypotensive, bradycardic, and renal sympathoinhibitory responses to a fixed-rate blood withdrawal in conscious, unrestrained rats. At least 24 h after electrode and catheter implantation, the animals were connected to the blood pressure and nerve recording apparatus while they rested unrestrained in their home cage. The second arterial catheter was connected to a preweighed heparinized 12-ml syringe inserted into a Harvard infusion/withdrawal pump. A drug- or vehicle-filled injector was inserted into the central guide cannula. The animals were allowed to habituate for at least 2 h before the experiment. Fifteen minutes of baseline recording were taken, after which 40 μg (5 μl) of methysergide or vehicle were injected over 2 min. This dose was previously found to completely block the bradycardic response to a rapidly induced hypotensive hemorrhage in conscious rats (40). Fifteen minutes after drug injection, hemorrhage was initiated by the withdrawal of blood at a rate of 1.26 ml/min. Blood withdrawal was terminated after 6 min in control animals. Because the hypotensive response was delayed by methysergide administration, blood withdrawal was extended an additional 2 min in drug-treated rats to allow examination of the hypotensive response. After termination of the withdrawal, the blood-filled syringe was weighed to validate the rate of blood withdrawal. Data from the experiment were only included in the analysis if the actual volume of blood withdrawn differed from the expected volume (as determined by the rate and duration of withdrawal) by <3%. The animals were then given a bolus dose of hexamethonium chloride (30 mg/kg) to block effenter RSNA for determination of background noise. After conclusion of the experiment, proper LV cannula placement was determined by injecting a 1-μl volume of diluted (1:10) India ink into the LV using the
same injector as used in the study. The animals were killed, and the brains were removed and cut in coronal sections at the level of cannulation and again at the level of the pontomedullary cistern. Proper cannula placement was confirmed by the presence of ink throughout the ventricular system as determined by visual inspection. Only data from rats with proper cannula placement were included in the study.

**Experimental protocol 2: assessment of 5-HT2-receptor blockade in the sympatholytic response to hemorrhage.** This experiment was performed to determine if selective blockade of 5-HT2 receptors (i.e., 5-HT2A, 5-HT2B, and 5-HT2C) mimicked the effects of methysergide during hemorrhage. The procedures used were identical to those described in protocol 1 except that control animals were injected with saline and experimental animals were injected with 40 g (80 nmol) of the selective 5-HT2-receptor antagonist LY-53857. This dose was chosen based on the similar affinity and antagonist potency of LY-53857 and methysergide at 5-HT2 receptors (19, 14, 23).

**Experimental protocol 3: assessment of the effects of 5-HT1A-receptor stimulation on the sympatholytic response to hemorrhage.** This experiment was performed to determine if stimulation of central 5-HT1A receptors mimicked the effects of methysergide on the cardiovascular and sympathetic responses to hemorrhage. The same procedure was used as in protocol 1 except that a 20-g (60 nmol) dose of the selective 5-HT1A-receptor agonist 8-OH-DPAT was injected rather than methysergide. The 20-g dose was chosen based on preliminary tests indicating that this dose produced maximal cardiovascular effects (e.g., bradycardia) when injected into the LV. Hemorrhage was terminated after 8 min in 8-OH-DPAT-treated animals to initiate a significant hypotensive response.

**Experimental protocol 4: assessment of selective 5-HT1A-receptor blockade on the effects of 8-OH-DPAT during hemorrhage.** 8-OH-DPAT was shown to produce a significant delay in the hypotensive response to hemorrhage (see RESULTS). To verify that the observed effect of 8-OH-DPAT was due to activation of 5-HT1A receptors, the selective 5-HT1A-receptor antagonist WAY-100635 was given before 8-OH-DPAT injection. The same procedures as outlined in protocol 3 were followed except that WAY-100635 (10 g or 20 nmol iv) was given 15 min before 8-OH-DPAT injection. In preliminary studies, this dose of antagonist was found to be the lowest dose that consistently blocked the cardiovascular effects of 8-OH-DPAT (20 g LV injection). An additional group of animals was given WAY-100635 (10 g iv) alone, 30 min before hemorrhage. Hemorrhage was terminated after 6 min in groups pretreated with WAY-100635.

**Experimental protocol 5: determination of the dose dependency of 8-OH-DPAT to delay the hypotensive response to hemorrhage.** Rats that appeared healthy but did not have viable nerves on the day of the experiment were treated as in protocol 4 except that the dose of 5-HT1A antagonist was varied (1-, 3-, 5-, and 10-g LV injections). The drug was diluted such that an equivalent volume (5 l) was delivered for each experiment. The average volume of blood withdrawn at the time blood pressure had fallen 40 mmHg from prehemorrhage baseline values was determined for each dose of 8-OH-DPAT. Data from experiments performed in protocol 3 (i.e., 20 g 8-OH-DPAT) were included in the analysis to provide a more complete dose-response curve.

**Experimental protocol 6: assessment of the effects of 5-HT1B/1D-receptor activation on the cardiovascular and sympathetic responses to hemorrhage.** This protocol was performed to determine whether activation of 5-HT1B/1D receptors by the selective 5-HT1B/1D-receptor agonist CGS-12066 mimicked the effects of methysergide during hemorrhage. The same procedure was performed as in protocol 1 except that 40 g of CGS-12066 (89 nmol) were LV injected before hemorrhage.

**Experimental protocol 7: assessment of the effects of 5-HT1A-receptor blockade on the ability of methysergide to attenuate the sympatholytic response to hemorrhage.** The same procedure was followed as in protocol 1 except that WAY-100635 (10, 30, or 100 g iv) was given 15 min before central methysergide injection. In these experiments, blood withdrawal was continued for 8 min after either the 10- or 30-g dose of antagonist but was terminated after 6 min in animals given 100 g of the antagonist.

**Data Analysis**

Blood pressure, HR, and RSNA values were averaged over 30-s blocks. A two-way ANOVA with repeated measures was used to determine the effects of drug on MAP, HR, and RSNA responses beginning 30 s before the start of blood withdrawal through 6 min of hemorrhage. The last 2 min of data collected from animals subjected to 8 min of hemorrhage were not subjected to between-group comparisons. Significant interactions between drug and time were followed up with post hoc tests to compare group means at each time interval from 30 s before the onset of hemorrhage through 6 min of blood withdrawal. The results were adjusted for repeated measures (see below). Unpaired, adjusted Student’s t-tests were used when two groups were compared. Bonferroni post hoc tests were used when three or more groups were compared. Adjusted, paired Student’s t-tests were used to make within-group comparisons between prehemorrhage baseline values and values obtained at each 30-s time interval throughout blood withdrawal. P values of <0.05 were accepted as significant for ANOVA. The acceptable significance of P values for between- and within-group post hoc tests was adjusted using the Dunn-Šidák method to control for repeated comparisons, i.e., using the equation P = 1 − (1 − P)K with P equal to the acceptable preadjustment level of significance (i.e., 0.05), P equal to the adjusted acceptable level of significance, and K equal to the number of comparisons (26). As described, some groups were subjected to an additional 2 min of blood withdrawal (i.e., 4 extra comparisons). Therefore, the acceptable significant P values for within-group comparisons was based on the larger number of comparisons for all treatment groups in order that the stringency to reach significance was not different between groups.

One-way ANOVAs were used to assess the effect of drug dose on the volume of blood withdrawal that produced a 40-mmHg fall in blood pressure. A dose-response curve for data from protocol 5 was constructed using a least-squares regression analysis to fit the data to a four-parameter logistic function with Sigma Plot (Version 2.01). The half-maximal effective dose (ED50) was calculated from the resulting best-fit curve. One-way ANOVAs were also used to assess the ability of the 5-HT1A-receptor antagonist to reverse the effect of methysergide on the change in MAP, HR, and RSNA during 6 min of hemorrhage.

**RESULTS**

**Response To Fixed-Rate Hemorrhage After Vehicle or Methysergide Injection**

Figure 1 demonstrates typical changes in MAP, HR, and RSNA to a fixed-rate hemorrhage (1.26 ml/min) in individual rats given LV injection of either vehicle (Fig.
Central injection of methysergide (40 μg/5 μl, Fig. 1B) 15 min before hemorrhage. Summary data for each treatment group are shown in Fig. 2. Baseline and hemorrhage responses in vehicle (n = 3)- and saline-injected animals (n = 4) were identical. Therefore, data from the two groups were pooled to provide control data for all experiments. In control experiments, hemorrhage produced a biphasic response. In the first phase, blood pressure was well maintained during the initial 3.5 min of blood withdrawal. This nonhypotensive phase of hemorrhage was accompanied by a significant rise in HR and RSNA. This initial phase was followed by a sudden fall in pressure beginning after ~4 min of blood withdrawal. The hypotensive response occurred simultaneously with a fall in HR and RSNA, although RSNA did not fall significantly below the original baseline. HR showed a dramatic fall that remained significantly lower than baseline throughout the remainder of hemorrhage.

Central injection of methysergide significantly lowered baseline HR and elevated baseline RSNA as shown by the significant difference between groups before the start of hemorrhage in Fig. 2. After injection of methysergide, bradycardia developed slowly, simultaneously with the onset of hindlimb abduction, a behavior characteristic of 5-HT1A-receptor stimulation (42). In contrast to controls, the methysergide-treated group showed only a slight, nonsignificant tachycardia during the initial phase of hemorrhage. Methysergide also appeared to block the sudden fall in MAP, HR, and RSNA observed in control animals subjected to hemorrhage. Instead, MAP began to decline slowly after ~5 min of blood withdrawal. RSNA only began to fall after 6.5 min of blood withdrawal. After 8 min of hemorrhage, the methysergide-treated group began to show a decline in HR. However, the response did not reach significance (Fig. 2).

Effect of 5-HT2-Receptor Blockade

LV administration of the 5-HT2-receptor antagonist LY-53857 significantly increased baseline RSNA (Fig. 3), an effect characteristic of 5-HT2-receptor blockade in the anesthetized rat (31). Despite the sympathoexcitatory effect of 5-HT2 blockade, the hemorrhage-induced cardiovascular responses of drug-treated animals did not differ from control animals (Fig. 3).

Effect of 5-HT1A-Receptor Stimulation

Central administration of the 5-HT1A-receptor agonist 8-OH-DPAT produced a pronounced behavioral effect consisting of the classic 5-HT1A receptor-mediated hindlimb abduction (42). The onset of the behavioral response occurred simultaneously with a decrease in blood pressure and HR. During the initial phase of hemorrhage, 8-OH-DPAT-treated rats showed a significant sympathoexcitatory response accompanied by a slowly developing tachycardic response that did not reach significance until after 5.5 min of hemorrhage. In contrast, control animals showed a significant tachycardic response after just 1.5 min of blood withdrawal. Similarly, the initial rise of RSNA developed more slowly in 8-OH-DPAT rats compared with controls. However, the maximal sympathoexcitatory response
eventually achieved by 8-OH-DPAT-treated rats was significantly greater than control animals (241 ± 32 vs. 153 ± 19% baseline, \( P < 0.05 \)). As seen in animals treated with methysergide, 8-OH-DPAT-treated rats showed a delayed hypotensive response to hemorrhage compared with controls. In addition, the tachycardic response to hemorrhage persisted throughout the duration of hemorrhage, whereas the onset of the sympathetic inhibitory and hypotensive responses to hemorrhage were no longer coupled in 8-OH-DPAT-treated rats. Specifically, sympathetic activity continued to rise during the beginning of hypotension and only began to decline slowly after 6 min of hemorrhage (Fig. 4). Systemic administration of the 5-HT\textsubscript{1A} antagonist WAY-100635 (10 \( \mu \)g iv) completely blocked behavioral responses to the 20-\( \mu \)g dose of 8-OH-DPAT (data not shown). The antagonist also reversed the cardiovascular effect of 8-OH-DPAT administration as demonstrated by the lack of difference in prehemorrhage MAP and HR between animals treated with vehicle and those given the 5-HT\textsubscript{1A}-receptor antagonist before 8-OH-DPAT injection (Fig. 4). Antagonist administration also completely reestablished the normal biphasic responses of MAP, HR, and RSNA during hemorrhage.

Administration of 10 \( \mu \)g WAY-100635 alone had no effects on the MAP, HR, or RSNA response to hemorrhage (Fig. 5).

Dose Dependency of 8-OH-DPAT to Delay Hypotensive Response to Hemorrhage

8-OH-DPAT dose-dependently increased the volume of blood withdrawal necessary to produce a 40-mmHg drop in blood pressure (Fig. 6). In control animals, blood pressure dropped by 40 mmHg after withdrawal of 5.6 ± 0.2 ml of blood. At the maximum dose of 8-OH-DPAT tested (20 \( \mu \)g), withdrawal of 9.2 ± 0.3 ml of blood produced the same fall in blood pressure, an increase of 64\% \( (P < 0.01) \). The calculated ED\textsubscript{50} for the response was 5.9 \( \mu \)g. Prior administration of WAY-100635 (10 \( \mu \)g) completely reversed the effects of the 20-\( \mu \)g dose of 8-OH-DPAT (5.4 ± 0.3 ml).

Effect of 5-HT\textsubscript{1B/1D}-Receptor Stimulation

All rats treated with the selective 5-HT\textsubscript{1B/1D}-receptor agonist CGS-12066 showed signs of increased alertness and activity within 2–3 min after injection consistent with the locomotor stimulatory effects attributed to 5-HT\textsubscript{1B}-receptor agonist administration (9).
However, the drug had no significant effect on baseline parameters or on responses to hemorrhage (Fig. 7).

Effect of 5-HT<sub>1A</sub>-Receptor Antagonist Administration on the Response to Methysergide During Hemorrhage

WAY-100635 administration dose dependently increased baseline sympathetic activity [10 μg: 28 ± 10%, n = 6; 30 μg: 40 ± 14%, n = 5; 100 μg: 65 ± 7% (P < 0.01), n = 4]. In addition, the drug dose dependently reversed the ability of methysergide to increase the volume of blood withdrawal that caused a 40-mmHg drop in blood pressure (Fig. 8, P < 0.01). As can be seen in Fig. 9, the 5-HT<sub>1A</sub> antagonist also dose dependently reversed the ability of methysergide to attenuate the hypotensive, bradycardic, and sympatholytic response to 6 min (i.e., 7.6 ml) of blood withdrawal.

DISCUSSION

The present study demonstrated that LV administration of the nonselective serotonin receptor ligand methysergide, as well as the 5-HT<sub>1A</sub>-receptor agonist 8-OH-DPAT, delayed the hypotensive, bradycardic, and renal sympathoinhibitory responses to fixed-rate hemorrhage in the conscious rat. The ability of 8-OH-DPAT to delay the hypotensive response to hemorrhage was dose dependent. In addition, the highly potent and selective 5-HT<sub>1A</sub>-receptor antagonist WAY-100635 reversed the effects of both 8-OH-DPAT and methysergide. Neither the 5-HT<sub>2</sub> antagonist LY-53857 nor the 5-HT<sub>1B/1D</sub> agonist CGS-12066 had any effect on responses to hemorrhage. This study is the first definitive demonstration that the ability of methysergide to attenuate the hypotensive bradycardic and sympathoinhibitory responses to hemorrhage is mediated by an agonist action on 5-HT<sub>1A</sub> receptors.

Methysergide is an ergoline derivative with potent serotonergic receptor antagonist properties, particularly on 5-HT<sub>2</sub> receptors (23, 32). However, several studies indicate that methysergide also activates a subset of 5-HT<sub>1</sub> receptors. Specifically, the methysergide-mediated vasoconstriction observed in several vessel types of various species has been attributed to an agonist effect on 5-HT<sub>1D</sub> and 5-HT<sub>1B</sub> receptors (7, 11, 20, 21, 43). In addition, second messenger assays have shown methysergide to be a relatively potent (log...
EC$_{50}$ = 6.4) and fully effective 5-HT$_{1A}$-receptor agonist (36). In a previous study, we found that the lowest dose of methysergide sufficient to delay the hypotensive response to hemorrhage also produced hindlimb abduction and lowered baseline blood pressure and HR, effects characteristic of 5-HT$_{1A}$-receptor activation (18). In the present study, the effect of methysergide on hemorrhage-induced cardiovascular responses was dose dependently eliminated by prior treatment with WAY-100635, a recently developed, highly potent, and selective 5-HT$_{1A}$-receptor antagonist without inverse agonist activity (17). Together, these data support the hypothesis that methysergide attenuates hemorrhage responses through stimulation of 5-HT$_{1A}$ receptors.

Methysergide is a potent antagonist of the 5-HT$_2$ receptor family. In this study, the persistent sympathoexcitatory response to methysergide was mimicked by the selective 5-HT$_2$-receptor antagonist LY-53857 but not the 5-HT$_{1A}$ agonist 8-OH-DPAT. These findings suggest that the initial sympathoexcitatory response to methysergide was due to blockade of 5-HT$_2$ receptors and was unrelated to the 5-HT$_{1A}$-mediated effects of the drug. Similar sympathoexcitatory responses to 5-HT$_2$-receptor blockade have been observed in anesthetized animals (31). In contrast to methysergide, LY-53857 had no effect on the cardiovascular and sympathetic responses to hemorrhage. In a previous study, Evans and colleagues (15, 16) found that LY-53857 was equipotent to methysergide in preventing the hypotensive response to central hypovolemia in conscious rabbits. However, these authors found a poor correlation between the potency of various ligands to prevent hypovolemia-induced hypotension and their respective affinities for rabbit 5-HT$_2$ receptors. Instead, LY-53857 was found to have some affinity for 5-HT$_{1A}$ receptors, suggesting that the drug may have stimulated 5-HT$_{1A}$ receptors to alter hypovolemia-induced responses (15). The apparent contradiction between the literature and the present study may have resulted from a species difference in the potency of LY-53857 at 5-HT$_{1A}$ receptors.

The possibility that 5-HT$_{1B}$- or 5-HT$_{1D}$-receptor activation contributes to the protective effect of methysergide during hemorrhage seems unlikely because methysergide has only limited intrinsic activity at these receptors (37). In the present study, CGS-12066, a more potent and efficacious 5-HT$_{1B}$- and 5-HT$_{1D}$-receptor agonist than methysergide, had no effect on either baseline or hemorrhage-induced cardiovascular responses when given at a slightly higher dose than methysergide (23, 37, 38). However, in vitro studies have shown that CGS-12066 and methysergide are equally potent and fully efficacious 5-HT$_{1A}$-receptor agonists (23, 36, 38). Therefore, it is surprising that CGS-12066 did not produce 5-HT$_{1A}$-mediated behav-

![Fig. 6. Dose-response curve relating the LV dose of 8-OH-DPAT given 15 min before hemorrhage and the volume of blood withdrawn when blood pressure had fallen 40 mmHg below prehemorrhage baseline. Nos. are shown in brackets above symbols. Additional data are shown for a single group given WAY-100635 (10 µg iv) 15 min before 8-OH-DPAT (20-µg LV injection). Data are group means ± SE. Body weights for rats given 8-OH-DPAT: 1 µg = 392 ± 3 g; 3 µg = 398 ± 3 g; 5 µg = 386 ± 4 g; 10 µg = 386 ± 4 g; 20 µg = 386 ± 4 g; 20 µg 8-OH-DPAT + 10 µg WAY-100625 = 397 ± 4 g. The EC$_{50}$, calculated from best fit of the dose-response curve, was 18 nmol.

![Fig. 7. Response of MAP, HR, and RSNA during fixed-rate hemorrhage in conscious, unrestrained rats 15 min after LV injection of vehicle (control, n = 7) or CGS-12066 (40 µg, n = 5 for MAP and HR, n = 4 for RSNA, BW = 390 ± 5 g). Data are group means ± SE. Between-group comparisons were made with Dunn-Sidak-adjusted unpaired t-tests. Within-group comparisons were made between prehemorrhage baseline and each subsequent interval with Dunn-Sidak-adjusted paired t-tests. Open symbols indicate significant within-group difference from prehemorrhage baseline, P < 0.05.](http://ajpregu.physiology.org/)
ioral effects or influence hemorrhage responses when given at a higher dose than methysergide. Interestingly, several studies have documented the ability of 5-HT₂-receptor blockade to enhance neuronal responses to 5-HT₁₆-receptor stimulation (4, 6). Given that CGS-12066 has only very limited affinity for 5-HT₂ receptors and only a negligible effect on 5-HT₂ receptor-mediated responses, it seems likely that the ability of methysergide to produce observable 5-HT₁₆-mediated effects may be dependent on a concurrent blockade of 5-HT₂ receptors (23, 38).

The possibility that other serotonergic receptor subtypes might have contributed to the effects of methysergide during hemorrhage was not tested in the present study. Methysergide shows high affinity for human recombinant 5-HT₅₆ and 5-HT₅₁ receptors. Though it is not clear whether these receptors are expressed in the rat brain, it is apparent that 8-OH-DPAT has little affinity for either receptor, suggesting that they have little role in the delay of the hemorrhage-induced cardiovascular responses observed in the present study (1, 2, 25, 45). The role of 5-HT₃ and 5-HT₄ receptors was not tested in this study, because methysergide has very low affinity for these receptors (8, 24, 27). Methysergide does bind rat recombinant 5-HT₅₆, 5-HT₅₁, and 5-HT₅₆ receptors with high affinity. However, these receptors are insensitive to 8-OH-DPAT, suggesting that they have no role in hemorrhage responses observed in the present study (5, 13). Methysergide also has antagonist properties at 5-HT₇ receptors (33, 44). However, it is unlikely that the effects of methysergide were due to blockade of endogenous 5-HT₇-receptor activation, because 8-OH-DPAT, a potent agonist of 5-HT₇ receptors, had no effect on hemorrhage responses after selective 5-HT₁₆-receptor blockade (11).

8-OH-DPAT delayed the response to hemorrhage at a lower dose than methysergide. This is not surprising given that 8-OH-DPAT is a more potent 5-HT₁₆-receptor agonist than methysergide (8.2 vs. 6.4 log EC₅₀) (36). Moreover, a 10-fold higher dose of WAY-100635 was needed to completely block the effects of methysergide than was needed to block the effects of 8-OH-DPAT, suggesting that a larger degree of 5-HT₁₆-receptor binding was necessary for methysergide to delay the hypotensive response to hemorrhage. The 40-μg dose of methysergide produced a similar, but slightly reduced, delay in the hypotensive response to hemorrhage compared with the 20-μg dose of 8-OH-DPAT. However, the same dose of methysergide completely suppressed the tachycardic response to blood withdrawal, whereas 8-OH-DPAT only partially sup-

![Fig. 8. Blood volume withdrawn at the time blood pressure had fallen 40 mmHg from prehemorrhage baseline values in animals subjected to a fixed-rate hemorrhage (1.26 ml/min). Animals were pretreated with LV injection of saline or vehicle (control), LV injection of 40 μg methysergide (Met), or LV injection of Met + increasing intravenous doses of WAY-100635 (WY). Data are group means ± SE. Group nos. are shown in brackets. One-way ANOVA was highly significant, P < 0.01. Group mean comparisons made with a Bonferroni post hoc test, #P < 0.05 compared with control, *P < 0.05 compared with Met, †P < 0.05 compared with Met + 10 μg WY.](http://ajpregu.physiology.org/)

![Fig. 9. ΔMAP, ΔHR, and ΔRSNA between prehemorrhage baseline and values determined after 6 min of hemorrhage. Animals were pretreated with LV injection of saline or vehicle (control), LV injection of 40 μg Met, or LV injection of Met + increasing intravenous doses of WY. Data are group means ± SE. Group nos. are shown in brackets. One-way ANOVA was significant for each parameter (ΔMAP and ΔHR, P < 0.01; ΔRSNA, P < 0.05). Group mean comparisons made with a Bonferroni post hoc test, **P < 0.01, *P < 0.05 compared with control, ##P < 0.01, #P < 0.05 compared with Met.](http://ajpregu.physiology.org/)
pressed this response. The possibility that activation of 5-HT$_{1A}$ receptors mediated the attenuated tachycardic response to hemorrhage is consistent with evidence that 5-HT$_{1A}$-receptor stimulation decreases cardiac sympathetic tone and increases cardiac parasympathetic drive (19). Such an effect could potentially override the baroreflex-mediated tachycardic response during the initial phase of hemorrhage. It is possible that methysergide provided a more complete suppression of the tachycardic response to hemorrhage by stimulating a larger 5-HT$_{1A}$ receptor-mediated response than 8-OH-DPAT. However, the latter drug produced a more pronounced bradycardia than did methysergide, suggesting that 8-OH-DPAT produced a larger 5-HT$_{1A}$ receptor-mediated effect on cardiac autonomic tone. It is more likely then that methysergide suppressed the tachycardic response to hemorrhage by effects on more than one receptor subtype. However, results of this study do not provide evidence as to what receptor subtypes, other than the 5-HT$_{1A}$ receptor, may have contributed to the effect.

Whereas the current study suggests that 5-HT$_{1A}$-receptor activation delays the hypotensive response to hemorrhage, what remains in doubt is the location of the receptors responsible for the effect. Scrogin et al. (40) showed that a central dose of methysergide, comparable with that used in the present study, had no effect on hemorrhage responses when given systemically. This evidence, together with that provided by the present study, suggests that activation of 5-HT$_{1A}$ receptors within the central nervous system likely mediates the delayed compensatory response to severe hemorrhage. 5-HT$_{1A}$ receptors are expressed presynaptically on the soma and dendrites of serotonergic neurons as well as postsynaptically throughout the brain and spinal cord. Stimulation of presynaptic 5-HT$_{1A}$ receptors reduces the release of serotonin from axon terminals (30). Therefore, the possibility remains that 5-HT$_{1A}$ agonists delay the hypotensive response to hemorrhage by attenuating endogenous serotonin release. Whereas this notion is consistent with evidence that serotonin depletion attenuates the hypotensive and sympatholytic response to hemorrhage in anesthetized rats and cats, it is contradicted by indications that serotonin depletion has no effect on hemorrhage responses in conscious rabbits (12, 16, 28). As yet, this controversy remains to be resolved. However, our findings that 5-HT$_{1A}$-receptor blockade did not accelerate the hemorrhage-induced fall in sympathetic activity suggests that postsynaptic 5-HT$_{1A}$-receptor activation, rather than decreased endogenous serotonin release, accounts for the observed effects of exogenous 5-HT$_{1A}$-receptor agonist administration during hemorrhage.

Significant cardiovascular depressor effects including hypotension, bradycardia, and sympathoinhibition, are elicited by postsynaptic 5-HT$_{1A}$-receptor activation in the hindbrain (18, 22, 29). In contrast, tachycardic, hypertensive, and sympathoexcitatory responses have been found with low-dose forebrain administration of 8-OH-DPAT (3). In our own preliminary studies, injection of the drug into the LV produced a biphasic response consisting of initial pressor, tachycardic, and sympathoexcitatory responses followed by bradycardia, mild hypotension, and sympathoinhibition (39). This profile of responses suggests that the early pressor effects are due to excitation of forebrain structures near the site of injection that are eventually overwhelmed by an inhibitory effect of hindbrain receptor activation after drug diffusion. Our work indicates that the initial excitatory responses (data not presented) as well as the subsequent depressor effects of 8-OH-DPAT are due to 5-HT$_{1A}$ activation, because all were blocked by the selective 5-HT$_{1A}$-receptor antagonist WAY-100635. Nevertheless, the data presented here do not provide evidence as to which set of responses, if either, is related to the ability of methysergide or 8-OH-DPAT to attenuate the hypotensive response to hemorrhage.

The efferent hemodynamic mechanisms that account for the delayed hypotensive response to hemorrhage after 5-HT$_{1A}$-receptor stimulation are unknown. The continued increase in renal sympathetic activity observed during hemorrhage in 5-HT$_{1A}$-receptor agonist-treated rats could have supported blood pressure by augmenting renin release and thus increased angiotensin II- and vasopressin-mediated vascular resistance. Alternatively, the rise in renal sympathetic activity may have been accompanied by an increased sympathetic drive to other vascular beds resulting in sympathetic-mediated preservation of peripheral resistance. Presently, it is unclear whether 5-HT$_{1A}$-receptor stimulation affects vasoactive peptide release or sympathetic tone in other vascular beds during the process of hemorrhage.

In summary, the present study provides unique evidence that the well-established ability of the nonselective serotoninergic ligand methysergide to attenuate the hypotensive and sympatholytic responses to hemorrhage in rats is due to activation of 5-HT$_{1A}$ receptors.

**Perspectives**

The notion that endogenous serotonin release mediates, at least in part, the sympatholytic response to hemorrhage and other sympatholytic pathologies has been discussed for many years (35, 41). This notion is based primarily on evidence that methysergide blocks the rapid sympatholytic effect of hemorrhage and that a similar effect is produced by serotonin depletion in anesthetized animals (12, 15, 28, 40). A previous study by Evans et al. (15) has already shed some doubt on the possibility that depletion of endogenous serotonin delays the sympatholytic response to hemorrhage in conscious animals. Our present findings that the effect of methysergide during hemorrhage is mediated by an agonist action on 5-HT$_{1A}$ receptors and that 5-HT$_{1A}$-receptor blockade has virtually no effect on hemorrhage further contradict the hypothesis that endogenous serotonin release mediates the rapid onset of hypotension during severe hemorrhage. Though this study would seem to rule out a role for endogenous serotonin release in the decompensatory response to.
hemorrhage, it does indicate that 5-HT1A-receptor activation can profoundly influence autonomic control of blood pressure, particularly during hypovolemia. What remains to be determined is what role, if any, endogenous activation of these receptors plays in the regulation of blood pressure and blood volume. Moreover, it is of interest to determine whether the pharmacological phenomenon described here can be exploited as a potential treatment in circulatory shock or other pathologies associated with loss of vascular sympathetic tone.

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