Role of the arterial baroreflex in 5-HT$_{1A}$ receptor agonist-mediated sympathoexcitation following hypotensive hemorrhage

Patrick Osei-Owusu and Karie Scrogin

Department of Pharmacology and Experimental Therapeutics, Loyola University Chicago, Stritch School of Medicine, Maywood, Illinois

Submitted 14 September 2005; accepted in final form 24 December 2005

Osei-Owusu, Patrick, and Karie Scrogin. Role of the arterial baroreflex in 5-HT$_{1A}$ receptor agonist-mediated sympathoexcitation following hypotensive hemorrhage. Am J Physiol Regul Integr Comp Physiol 290: R1337–R1344, 2006. First published January 5, 2006; doi:10.1152/ajpregu.00671.2005.—5-HT$_{1A}$-receptor agonists rapidly restore blood pressure and sympathetic activity in conscious rats subjected to hypotensive hemorrhage. 5-HT$_{1A}$-receptor activation has also been shown to produce a robust increase in baroreceptor-dependent, pulse-synchronous firing of cardiac sympathetic nerves in anesthetized cats. To determine whether 5-HT$_{1A}$-receptor agonists reverse hemorrhage-induced suppression of sympathetic activity through facilitation of the arterial baroreflex, the effects of the 5-HT$_{1A}$-receptor agonist, 8-OH-DPAT, were assessed in male Sprague-Dawley rats subjected to sinoaortic baroreceptor denervation and subsequent hypotensive hemorrhage. 8-OH-DPAT produced rapid pressor and sympathoexcitatory responses in hemorrhaged animals that were attenuated, but not blocked, by sinoaortic denervation (SAD) (+49 ± 4 vs. +37 ± 4 mmHg; +165 ± 30 vs. +92 ± 24% baseline, P < 0.01).

Spectral analysis of sympathetic activity showed that SAD abolished the 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT)-mediated increases in pulse-synchronous (13 ± 1 vs. 5 ± 1% total power for intact vs. SAD rats, P < 0.01) and Mayer wave-related bursting (18 ± 3 vs. 8 ± 1% total power, P < 0.05). However, 8-OH-DPAT continued to increase total power (+72 ± 22 vs. −63 ± 7% prehemorrhage total power, P < 0.05) and power at the respiratory frequency (35 ± 2 vs. 25 ± 4% total power) in SAD animals. These data indicate that full expression of the sympathoexcitatory effect of 8-OH-DPAT requires a functional arterial baroreflex. However, a portion of the effect is due to activation of arterial baroreflex-independent sympathetic pathways.

DURING MILD BLOOD loss, compensatory increases in sympathetic-mediated vascular resistance help to maintain blood pressure (BP). The increase in sympathetic activity that occurs during the initial phase of hemorrhage has been attributed to activation of the arterial baroreflex (24). With continued blood loss (15–30% of blood volume), arterial baroreflex-mediated compensation is overridden by some unknown stimulus and sympathetic activity rapidly becomes suppressed leading to a sharp drop in BP (17, 37). Previously, we showed that systemic administration of 5-HT$_{1A}$-receptor agonists, given immediately after the onset of the hypotensive phase of hemorrhage, reverses the sympathoinhibitory, bradycardic, and hypotensive responses to blood loss in the conscious rat (35). We also showed that blockade of the autonomic nervous system prevented the 5-HT$_{1A}$ receptor agonist-mediated pressor response (30), indicating that the rapid reversal of hypotension was due to sympathetic-mediated vasoconstriction. However, the mechanism through which 5-HT$_{1A}$-receptor agonists elicit sympathoexcitation during severe hypotensive hemorrhage is not known.

5-HT$_{1A}$-receptor agonists stimulate a robust increase in pulse-synchronous bursting of cardiac sympathetic nerves in euvolemic, anesthetized cats (29). This effect is prevented by sinoaortic denervation (SAD). We observed a similar effect on pulse-synchronous renal sympathetic activity in conscious, hypovolemic rats (P. Osei-Owusu, unpublished observations). Therefore, we speculated that 5-HT$_{1A}$-receptor agonists raise sympathetic activity during severe hemorrhage by reversing suppression of arterial baroreflex function. This hypothesis was tested by determining whether the sympathoexcitatory effect of 5-HT$_{1A}$-receptor agonist administration in hypovolemic rats was blocked by abolition of the arterial baroreflex.

METHODS

Animals

Male Sprague-Dawley rats weighing between 350 and 400 g (Harlan, Indianapolis, IN) were given ad libitum access to food and water. The rats were acclimated to the housing facility for at least 1 wk before surgery. The facility was maintained at a constant temperature of 22°C with a 12:12-h light-dark cycle. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the U.S. National Institutes of Health.

Surgery

Sinoaortic denervation. Chronic SAD was performed as described previously with some modifications (18). Briefly, rats were injected with the anti-sialogogue, glycopyrolate (0.5 mg/kg ip), 5 min after anesthesia (100 and 7 mg/kg ip ketamine/xylazine). The rats were intubated and mechanically ventilated with room air. The carotid sinus was exposed through a ventral midline neck incision. The aortic depressor, superior laryngeal, and carotid sinus nerves were bisected, after which the glossopharyngeal nerve was carefully cleaned of all extraneous fibers. The same procedure was repeated on the opposite side. Following surgery, the rats received once daily subcutaneous injections of 5% dextrose (10–15 ml) for 4 or 5 days to maintain hydration. In sham-operated animals, the carotid sinus was exposed briefly before closure of the incision. The animals were allowed at least a 2-wk recovery before further surgical intervention.

Renal nerve electrode and vascular catheter implantation. Twenty four hours before the experiment, rats were anesthetized with pentobarbital sodium (65 mg/kg ip) and implanted with bilateral femoral
arterial catheters, as well as a unilateral femoral venous catheter (PE-50 heat-welded to a length of PE-10) to enable direct measurement of arterial pressure, blood withdrawal, and drug injection, respectively. 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. The electrode connector was externalized subcutaneously at the nape of the neck along with the vascular catheters. Viability of the nerve preparation was determined by visual assessment of nerve activity, after which the preparation was embedded in lightweight dental silicon (Bisico S4i, Bisico, 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 overnight in their home cage.

**Data Acquisition**

During all experiments, arterial pressure, heart rate (HR), and renal sympathetic nerve activity (RSNA) were recorded continuously on a Macintosh G4 Powerbook computer using PowerLab data acquisition software (Chart v. 3.6.1, ADInstruments, Colorado Springs, CO). Arterial pressure was measured with a disposable pressure transducer (Transpac IV, Abbott Labs, North Chicago, IL) and a PowerLab bridge amplifier (ADInstruments). HR was calculated using peak-to-peak detection of the pulse pressure wave. Sympathetic activity was filtered (1–1,000 Hz), sampled (2,000 Hz), and amplified (√10–20,000) with a PowerLab Bioamplifier (ADInstruments). The recorded neurogram was full-wave rectified and integrated over a 20 ms time constant. Background noise in the electrode recording was determined at the end of each experiment by measuring the remaining signal following ganglionic blockade (30 mg/kg iv hexamethonium chloride). Background noise was subtracted from nerve activity values to provide a measurement of RSNA. All measurements of RSNA were normalized to basal nerve activity (%baseline) determined over a 10-min period directly before hemorrhage. Only data from animals with greater than a 2:1 signal-to-noise ratio were included in the study.

**Experimental Design**

Before the experiment, careful assessment of the success of the SAD surgery was made by determining the HR responses to a single, submaximal dose of sodium nitroprusside (10 μg/kg iv). Because of the delayed response of the relatively sluggish sympathetic system, the maximal sympathetic response or maximal HR response was compared with the maximal BP response for determination of the baroreceptor-mediated response. Only those animals that showed a highly variable BP without significant reflex HR response to nitroprusside administration (HR barosensitivity lower than −1.5 beats·min⁻¹·mmHg⁻¹) were included in the data analysis. Animals were connected to the recording instrumentation and withdrawal pump while resting unrestrained in their home cage. The intravenous catheters were flushed and connected to PE tubing filled with appropriate dose of drug or vehicle. One arterial line was connected to a syringe fitted on a withdrawal pump while the second was connected to the BP transducer. The recording electrode and all vascular catheters were connected to the instruments via an overhead swivel system to enable undisturbed recordings and blood withdrawal while the animals rested, unrestrained in its home cage. The rat was then allowed to rest for at least 2 h before the hemorrhage. Arterial pressure, HR, and RSNA were recorded continuously beginning 20 min before the hemorrhage and ending 20 min following hemorrhage termination. Controlled blood withdrawal was initiated at a rate of 3.2 ml·min⁻¹·kg⁻¹ for 6 min, after which the withdrawal speed was reduced to 0.53 ml·min⁻¹·kg⁻¹ for an additional 4 min. In previous work, this procedure was found to produce a consistent fall in mean arterial pressure (MAP), HR, and RSNA after withdrawal of ~11.2 ml/kg of blood or ~14% of estimated blood volume. The lower rate of withdrawal was found to be sufficient to maintain bradycardic and sympatholytic responses until termination of hemorrhage (30).

In both SAD and intact animals, the selective 5-HT₁A agonist, 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT; 30 nmol/kg iv), or saline was injected after establishment of hypotension, 7 min after the initiation of blood withdrawal. The 30-nmol/kg dose was chosen based on a previous study indicating it was the half maximal dose that prolonged the normotensive phase of hemorrhage when the drug was delivered 15 min before initiation of blood withdrawal (35). Twenty minutes after termination of hemorrhage, hexamethonium (30 mg/kg iv) was administered to assess background noise in the recording electrode.

**Data Analysis**

BP, HR, and RSNA were averaged over 1-min intervals. Data acquired during blood withdrawal before injection were pooled within the SAD and sham groups and analyzed with two-way ANOVAs to assess the effect of SAD on BP, HR, and RSNA responses to hemorrhage. Significant interactions between surgical intervention and time were followed up with Bonferroni post hoc tests with Dunn-Sidák correction of P values, each minute (23). To present the data over the course of the recording period, three-way ANOVAs with repeated measures were used to assess the effect of SAD and drug treatment on BP, HR, and RSNA in hemorrhaged rats over time. Preplanned comparisons between groups were performed at the time of injection and 10, 15, 20, 25, and 30 min after initiation of hemorrhage.

**Spectral analysis of RSNA**

Continuous 1-min segments of filtered, rectified, and integrated RSNA recorded before hemorrhage and 3 min after administration of 8-OH-DPAT or saline were used to generate RSNA power spectra. For each 1-min segment, 14 data sets of n = 16,384 points with 50% overlap were processed. After removal of linear trend and application of Hanning window, power spectra were generated using a fast Fourier transform algorithm (9, 10). The average spectrum was obtained from spectra generated from the 14 data sets with a frequency resolution of 0.144 Hz. Spectral power was quantified by integration within the following frequency bands: total power, 0 to 20 Hz; power at the Mayer wave frequency, 0.2 to 0.8 Hz; and power at the respiratory-related frequency, 0.8 to 2.5 Hz. Cardiac-related power was calculated as the power extending 0.5 Hz from the mean HR frequency averaged over the 1-min segment. Total power obtained 3 min after injection of saline or 8-OH-DPAT was calculated as a percentage of prehemorrhage total power. Power at the respiratory and cardiac-related frequencies, as well as power at the Mayer wave frequency, was calculated as a percentage of the total power 3 min after injection.

Two-way ANOVAs were used to determine the effect of SAD and drug treatment on total power, as well as power at the Mayer wave-, respiratory-, and cardiac-related frequencies. Tukey/Kramer post hoc analyses were used to determine group differences. P values <0.05 were considered statistically significant.

**RESULTS**

On the day of the experiment, SAD rats weighed 326 ± 6 g while sham-denervated rats weighed 363 ± 8 g (P < 0.01). Arterial baroreceptor denervation produced a 75–85% decrease in cardiac (−2.84 ± 0.21 vs. −0.72 ± 0.20 beats·min⁻¹·mmHg⁻¹, P < 0.01) and sympathetic baroreceptor-mediated responses (−3.09 ± 0.46 vs. −0.45 ± 0.15% RSNA/mmHg, P < 0.01).

BP was well maintained in sham-operated animals during the initial phase of hemorrhage and then fell sharply after the
third minute (9.6 ml/kg blood withdrawal; Fig. 1, A and B), reaching a nadir of 53 ± 2 mmHg after 6 min (19.7 ml/kg) of blood withdrawal (Fig. 2). In contrast, BP of SAD rats began to fall progressively after the first minute of blood withdrawal, followed by a more abrupt fall 2–3 min after the start of hemorrhage (Fig. 1 B). SAD rats achieved the same nadir in BP (51 ± 4 mmHg) as intact animals for the same rate and volume (normalized to body weight) of blood withdrawal (Fig. 2). HR was well maintained in both sham and SAD animals during the first 3 min of blood withdrawal (431 ± 13 vs. 430 ± 11 beats/min 3 min after start of hemorrhage). In sham-operated animals, sympathetic activity began to rise immediately after initiation of blood withdrawal and reached 126 ± 18% above baseline 3 min into the hemorrhage. Sympathetic activity rose by only 44 ± 7% of baseline in SAD animals at the same time point (P < 0.01 compared with sham). During the hypotensive phase, the fall in BP was paralleled by a large fall in HR (−126 ± 16 vs. −88 ± 10 beats/min) and sympathetic activity (−140 ± 14 vs. −54 ± 9% baseline, from 4 to 7 min after start of hemorrhage, P < 0.01) in sham-operated and SAD animals, respectively.
An overall ANOVA of BP obtained after drug injection showed significant main effects for drug treatment, surgical intervention, and time (P < 0.01, P < 0.05, and P < 0.01, respectively). As seen in Fig. 2, 8-OH-DPAT produced a rapid recovery of BP in sham-operated animals, whereas saline-treated animals showed a slower recovery of pressure during the first 10 min following termination of blood withdrawal. In SAD animals, 8-OH-DPAT injection also elicited a significant pressor response, although the effect was significantly attenuated compared with sham-operated rats treated with the agonist. The pressor response to 8-OH-DPAT was sustained throughout the posthemorrhage recording period in sham-operated animals. BP continued to recover throughout the recording period following the initial pressor response to 8-OH-DPAT in SAD rats. A tendency for a drug treatment × surgical intervention interaction did not reach significance (P = 0.068) because of the recovery of BP to similar levels in all groups during the last half of the recording period. However, a three-way ANOVA with repeated measures assessing BP at the time of injection and 3 min later showed a significant interaction resulting from the attenuated response to 8-OH-DPAT in SAD animals (P < 0.05).

An overall three-way ANOVA of the HR response demonstrated a main effect for time (P < 0.01). Neither drug treatment nor surgical intervention had a significant effect on HR.

An overall three-way ANOVA of RSNA demonstrated main effects of drug treatment, surgical intervention, and time (all P < 0.01). 8-OH-DPAT produced a robust increase in sympathetic activity in sham-operated animals. Although SAD rats given 8-OH-DPAT showed a significant recovery of sympathetic activity compared with SAD rats treated with saline, the response was attenuated compared with sham-operated animals (Fig. 2). The sympathoexcitatory effect in sham-operated rats persisted throughout the posthemorrhage recording period. In both sham-operated and SAD rats treated with saline, RSNA remained attenuated even after termination of hemorrhage, and it only began to rise slowly 5 min after the end of hemorrhage. However, the interaction between drug treatment and surgical intervention was not significant when assessed over the entire recording. A three-way ANOVA restricted to time of injection and 3 min after injection demonstrated a significant interaction between surgical intervention and drug treatment, again due to the attenuated sympathetic response to drug in sham-operated rats.

As can be seen in the individual prehemorrhage baseline power spectra of integrated RSNA in Fig. 3, three prominent peaks were distinguishable in the sham-operated animals: a low-frequency peak coincident with the Mayer wave oscillations in BP (0.2–0.8 Hz), a respiratory-related frequency peak (0.8–2.5 Hz), and a cardiac-related frequency peak (5.5–6.5 Hz). Baroreceptor denervation significantly reduced baseline power at the Mayer wave- and the cardiac-related frequencies and increased the percentage of power in the respiratory-related frequency in euvoletic animals before hemorrhage (Figs. 3 and 4). A two-way ANOVA assessing responses to drug after hemorrhage demonstrated significant main effects for surgical intervention and drug treatment (P < 0.05, P < 0.0001, respectively), but the interaction between the two factors did not reach significance. 8-OH-DPAT injection produced a significant increase in the total power in both hemorrhaged SAD-operated and sham-operated animals (Figs. 3 and 5) compared with saline. Two-way ANOVAs assessing power in the individual frequency bands showed a significant main effect of surgical intervention on cardiac- and Mayer wave-related power (P < 0.001, P < 0.05, respectively) but not on respiratory-related power. Main effects of drug treatment were found for respiratory-related power, but not cardiac- or Mayer wave-related power. Significant interactions between surgical intervention and drug treatment were observed for cardiac- and Mayer wave-related power. The interaction was due to the ability of 8-OH-DPAT injection to increase power in intact hemorrhaged animals, but not in barodenervated animals (Figs.

**Fig. 3.** Typical frequency spectra of integrated RSNA in individual sham-operated and SAD rats determined 5 min before hemorrhage and 3 min after 8-OH-DPAT injection.

**Fig. 4.** Summary data from spectral analyses of integrated RSNA of sham-operated and SAD rats during prehemorrhage baseline recording period. Power at the respiratory-, cardiac-, and Mayer wave-related frequencies calculated as a percentage of the prehemorrhage total power. Values are means ± SE. Between-group comparisons were performed using Student’s t-test. **P < 0.01 vs. sham.
3 and 5). In contrast, 8-OH-DPAT injection also significantly increased power at the respiratory-related frequency in both sham-operated and denervated rats (Figs. 3 and 5). The effect of 8-OH-DPAT is demonstrated more clearly in Fig. 6, in which sympathetic bursting is shown in conjunction with pulsatile and mean pressure. A noticeable increase in pulse-synchronous bursting occurred coincident with intermittent pressure nadirs (Mayer wave fluctuations) that developed approximately every 2.5 s (noted by ○) in the intact animal. In contrast, more rapid oscillations in pressure with respiration were visible in recordings from the SAD rat. There was also a predominance of bursting in time with respiratory-related fluctuations in BP (indicated by ●) in the SAD rat.

**DISCUSSION**

BP in SAD rats fell only after at least 1 min of blood withdrawal. This seemed somewhat surprising given previous evidence in rabbits that arterial baroreceptors control compensatory drive during blood loss (31). However, others have also seen short delays in the pressure fall during hemorrhage in barodenervated rabbits (32). Careful study of the individual experiments in this report suggests that the lack of an immediate fall in BP was due to the relatively large BP variability observed in denervated rats. Presumably, a sufficient amount of blood must be lost before the uncompensated fall in cardiac output overcomes the variability in BP. Alternatively, some compensation could conceivably arise from an alternative source such as unloading of cardiopulmonary stretch receptors. Indeed, Shreihofer et al. (33) observed an immediate and large fall in pressure in conscious rats subjected to chronic nucleus of the solitary tract (NTS) lesion following a small volume of blood withdrawal that was not sufficient to alter pressure in either control or chronic SAD rats. These data suggest the possibility that additional visceral afferent input maintains BP during hemorrhage.

Barodenervation also had unique effects on HR responses. Previous studies consistently showed a complete blockade of the initial tachycardia and late fall in HR that accompanies progressive blood loss in arterial baroreceptor-denervated rabbits. In contrast, we found no effect of SAD on the initial tachycardic response to blood loss in rats. In our study and others, the tachycardic response is typically very minor and often not significant in the conscious rat (7). Barodenervation also had no effect on the very profound bradycardic response that typically develops after severe hemorrhage in the conscious rat. This latter effect is speculated to result from paradoxical activation of cardiac ventricular mechanoreceptors (28). Although this view remains controversial, chronic lesion of the NTS but not arterial baroreceptor denervation also blocks the bradycardic response to hemorrhage in conscious rats, suggesting that some visceral afferents other than arterial baroreceptors trigger the response.

Intravenous administration of the selective 5-HT\textsubscript{1A}-receptor agonist, 8-OH-DPAT, rapidly increased BP, HR, and RSNA in conscious intact and arterial baroreceptor-denervated rats subjected to hypotensive hemorrhage. However, the pressor and sympathoexcitatory effects of 8-OH-DPAT were significantly attenuated in denervated rats. Frequency analysis of the renal sympathetic bursting pattern demonstrated an overall increase in total power (between 0 and 20 Hz) after 8-OH-DPAT injection in both denervated and intact animals. In sham-denervated rats, sympathetic bursting was entrained predominantly to the cardiac and respiratory cycles as well as to the Mayer wave fluctuations of BP (~0.4 Hz). 8-OH-DPAT significantly increased power at all three frequencies in intact rats.

**Fig. 5.** Summary data from spectral analyses of integrated RSNA of intact and SAD rats treated with 8-OH-DPAT (30 nmol/kg iv) or saline during severe hemorrhage. Total power, determined 3 min after drug or saline injection, was calculated as %change from prehemorrhage baseline (A). Cardiac (B), respiratory (C), and Mayer wave-related power was calculated as a percentage of the total power determined 3 min after saline or 8-OH-DPAT injection. Values are means ± SE. Between-group comparisons were performed using Tukey/Kramer post hoc tests. *,**P < 0.05, <0.01 vs. sham/saline; #, ##P < 0.05, <0.01 vs. SAD/saline; &, &&P < 0.05, <0.01 vs. SAD/8-OH-DPAT, respectively.

**Fig. 6.** Five-second segments of arterial pressure, mean pressure (superimposed on arterial pressure), and integrated RSNA from individual intact and SAD rats recorded 3 min after 8-OH-DPAT injection. Note increased pulse-synchronous bursting activity during the nadir of Mayer wave fluctuations in pressure, in intact animal (indicated by ○), and the predominance of respiratory-related bursting (●) with reduced pulse synchronicity in SAD rat.
Arterial baroreceptor denervation abolished 8-OH-DPAT-induced pulse-synchronous bursting and attenuated drug effects on bursting fluctuations at the Mayer wave frequency. In denervated rats, the increase in total power observed following 8-OH-DPAT injection was reflected primarily by increased bursting entrained to respiratory oscillations.

The sympathoexcitatory response to 8-OH-DPAT was not likely a secondary response to a peripheral pressor effect of the drug. In a previous study, the partial 5-HT1A-receptor agonist, buspirone, was found to exaggerate the sympathoexcitatory response in hypovolemic rats when the pressor response to drug was blocked with the α1-adrenergic receptor antagonist prazosin (30). Moreover, normalization of pressure has been found to further suppress sympathetic activity in conscious rabbits subjected to hypotensive hemorrhage (12). Together, the data suggest that 8-OH-DPAT increases sympathetic activity through a central site of action rather than through a peripheral pressor effect. In accordance, work from our laboratory showed that 8-OH-DPAT acts in the brain to rapidly restore BP when administered immediately after establishment of hypotensive hemorrhage in conscious rats (34). Ganglionic blockade prevents the pressor response to 8-OH-DPAT, indicating that the effect is due primarily to sympathetic-mediated vasoconstriction or sympathetic-mediated vasoactive hormone release (30).

The central nervous system site where 5-HT1A-receptor agonists act to increase sympathetic activity in hypovolemic animals is not known. Previous work by others (11, 21, 25, 27) demonstrated an almost exclusive sympathoinhibitory effect of 8-OH-DPAT in normovolemic animals. Such effects have been attributed to hyperpolarization of presympathetic neurons in the rostral ventrolateral medulla (13–15). To our knowledge, only two studies have reported sympathoexcitatory effects of 8-OH-DPAT. Both studies attributed the effect to activation of 5-HT1A receptors in the forebrain (2, 38). In contrast, we found that the sympathoexcitatory effect of 8-OH-DPAT was progressively more robust, and the latency to onset of effect shorter, with more caudal cerebroventricular administration. Injection of drug into the fourth ventricle produced the most rapid effect, suggesting a medullary hindbrain site of action (34).

Findings that baroreceptor denervation attenuated both 8-OH-DPAT-mediated sympathoexcitation and 8-OH-DPAT-dependent entrainment of sympathetic activity to the cardiac and Mayer wave frequencies suggest that facilitation of baroreflex control of sympathetic activity may, in part, mediate the sympathoexcitatory effect of 5-HT1A-receptor agonists in hypovolemia. A similar increase in pulse-synchronous activity of postganglionic cardiac fibers was observed following bilateral injection of 8-OH-DPAT into the lateral segmental field (LTF) of anesthetized, euvoicmic cats (3, 4). As in the present study, the 8-OH-DPAT-induced entrainment of sympathetic bursting to the cardiac cycle was abolished by prior baroreceptor denervation, suggesting that a site analogous to the LTF might be responsible for the baroreceptor-dependent sympathoexcitation produced by 8-OH-DPAT in the hypovolemic rat. In contrast to our study, 8-OH-DPAT did not affect total power in the baroreflex-intact, anesthetized cat (29). This contradiction may reflect a difference in drug effect on sympathetic output to cardiac and renal targets, or alternatively, it may suggest that facilitation of baroreflex function has a more profound effect on total sympathetic activity when the prevailing level of activity is low, as in our acute model of hypotensive hemorrhage.

The LTF is one of only a few regions that provide tonic excitatory input to the rostral ventrolateral medulla (rVLM) in the cat. Recent work suggests that the caudal portion of the caudal ventrolateral medulla provides excitatory input to the rVLM in the rat (26). Researchers have speculated that such a tonic excitatory projection from the cVLM may be analogous to the excitatory LTF input to the rVLM identified in cats (26). To date, no studies have so far assessed the effect of 5-HT1A-receptor activation in the pressor region of the cVLM.

There is only limited evidence that 5-HT1A-receptor agonists alter baroreflex function. In ventilated, anesthetized rats, a similar systemic dose of 8-OH-DPAT as used in the present study was shown to shift the baroreflex regulation of rVLM neuronal firing rate and lumbar sympathetic activity to lower pressures without influencing gain (27). It was not determined whether the baroreflex shift was due to baroreceptor resetting, as BP itself was reduced by drug treatment. Nevertheless, a shift of baroreflex control to lower pressures could not account for the baroreflex-dependent elevation of sympathetic drive in hypovolemic animals observed in the present study. Evidence suggests that the baroreflex shift to lower pressure is due to activation of 5-HT1A receptors in the rVLM. This would seem to rule out a role for rVLM 5-HT1A receptors in the 5-HT1A-receptor-dependent sympathoexcitatory phenomenon observed in hypovolemic animals.

In a previous study, the same dose of the partial 5-HT1A-receptor agonist, buspirone, that produced a robust sympathoexcitatory response in hypovolemic, hemorrhaged rats caused a significant sympathoinhibitory response in euvoicmic animals instrumented in the same manner (30). These data led us to speculate that 5-HT1A-receptor agonists may mediate their unique sympathoexcitatory effect through disinhibition of neurons that are normally quiescent in euvoicmic animals but active in hypovolemic animals. In accordance, 5-HT1A receptors are coupled to the Gi/Go α-subunit of heterotrimmeric G proteins which, when activated, typically inhibit adenyl cyclase, increase potassium conductance, or reduce calcium currents (1, 5, 6). In accordance, 5-HT1A-receptor stimulation typically leads to hyperpolarization of neurons (8). Thus it seems reasonable to suggest that a 5-HT1A-receptor-mediated inhibition of cells that suppress baroreflex function during acute hypotensive hemorrhage may account for the baroreflex-dependent sympathoexcitatory effect of 8-OH-DPAT.

Alternatively, the sympathoexcitatory response to 5-HT1A-receptor agonists may be masked by a concomitant sympathoinhibitory effect of stimulation of 5-HT1A receptors expressed by active rVLM neurons in euvoicmic animals. In contrast, 5-HT1A-receptor agonists would presumably have little effect on rVLM presympathetic neurons that are already suppressed by hypotensive hemorrhage. In this scenario, the sympathoexcitatory response to 5-HT1A-receptor agonists must ultimately be mediated by an alternative population of presympathetic neurons that do not express 5-HT1A receptors and thus are not sensitive to hyperpolarization by systemic administration of 5-HT1A-receptor agonists.

8-OH-DPAT continued to produce a robust increase in sympathetic activity in hemorrhaged animals subjected to arterial baroreceptor denervation. This was not likely due to...
incomplete denervation as SAD surgery completely blocked the 8-OH-DPAT-induced entrainment of RSNA to the cardiac cycle and the Mayer wave frequency, both of which are dependent on arterial baroreceptor innervation (19). Nevertheless, postinjection power at the cardiac or Mayer wave frequencies was not different in SAD and sham-operated animals treated with saline. As can be seen in the spectrum of SAD and sham-operated animals determined just before drug administration, there was only limited total power after hemorrhage. The lack of difference in postinjection cardiac-related power between SAD and intact saline-treated groups was likely due to the very low overall power in both groups, making statistical difference at a specific point very difficult to discern. Indeed, SAD significantly reduced power at the cardiac and Mayer wave frequencies when sympathetic activity was normal, before blood withdrawal.

Interestingly, in the present study, sympathetic bursting related to respiratory frequency was elevated in SAD rats. Previous work by others suggests that power of RSNA bursting at the respiratory frequency is largely dependent on an intact arterial baroreceptor reflex. However, sporadic expression of power at the respiratory frequency is seen under these conditions (19). Others, in contrast, have noted increased respiratory-related firing of sympathetic nerve following acute arterial baroreceptor denervation in anesthetized piglets (36). We found a consistent expression of respiratory-related bursting in both intact rats and those denervated 2 wk before the experiment. Qualitatively, the increased respiratory power appeared to be related to loss of power in the Mayer wave and cardiac frequencies rather than to an increase in absolute power at the respiratory cycle. However, between-animal comparisons of absolute power of RSNA are difficult to validate due to the variation in quality of the sympathetic recording preparations.

Nevertheless, removal of the baroreflex-dependent components of nerve bursting in 8-OH-DPAT-treated animals unmasked a robust increase in respiratory-related bursting. Injection of 8-OH-DPAT in the LTF of anesthetized, ventilated cats had no apparent effect on respiratory-related bursting, suggesting either that respiratory-related effects on sympathetic bursting are not mediated by the LTF or that mechanical ventilation or anesthesia masked the effect in the previous study (29). Taken together, the data indicate that the sympathoexcitatory effect of 8-OH-DPAT in SAD animals appears to be due, in part, to an additional baroreflex-independent mechanism, possibly through a direct effect on central respiratory drive or through facilitation of coupling between respiratory and presympathetic nuclei.

Carotid sinus nerve section blocks peripheral chemosensory afferent signaling. Therefore, alterations in peripheral chemosensation are not likely responsible for the effect of 8-OH-DPAT on respiratory entrainment of sympathetic activity. The effect of 5-HT1A receptor agonists on central chemosensation is not known. However, there is evidence for a direct role of 5-HT1A receptors in the regulation of respiratory oscillations at the level of the preBötzinger complex in normal animals (20). Whether 5-HT1A receptor agonists increase respiratory oscillation in sympathetic nerve discharge through direct effects on rhythm-generating cells of the preBötzinger nucleus remains to be determined. Nevertheless, it is tempting to speculate that 5-HT1A receptor agonists could serve as effective adjuvants to volume resuscitation in the treatment of circulatory shock, by virtue of their ability to augment respiratory drive and the concurrent increase in sympathetic activity. Indeed, resuscitation with increased oxygen typically improves prognostic indicators in recovery from circulatory shock (16, 22).

In summary, administration of 5-HT1A-receptor agonists produces arterial baroreflex-dependent sympathoexcitation in hypovolemic animals. Additional baroreflex-independent sympathoexcitatory responses to drug administration may be mediated by drug action at an independent site that regulates respiratory function. Together, these data provide further evidence that 5-HT1A-receptor agonists may be a useful adjunct to current methods of fluid resuscitation in the treatment of hypovolemic shock characterized by depression of sympathetic activity.

ACKNOWLEDGMENTS

The authors thank Dr. C. Webber for helpful comments during preparation of the manuscript.

GRANTS

This work was supported by National Institutes of Health Grants HL-72354 and HL-76162 to K. E. Scrogin and American Heart Association Grant 0310026Z to P. Osei-Owusu.

REFERENCES


