Neurosteroid modulation of arterial baroreflex-sensitive neurons in rat rostral ventrolateral medulla

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Laiprasert, J. D., R. C. Rogers, and C. M. Heesch. Neurosteroid modulation of arterial baroreflex-sensitive neurons in rat rostral ventrolateral medulla. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R903–R911, 1998.—The major metabolite of progesterone, 3α-OH-dihydroprogesterone (3α-OH-DHP), is the most potent endogenous positive modulator of central nervous system GABA<sub>A</sub> receptors. Acute intravenous administration of 3α-OH-DHP to virgin female rats potentiates arterial baroreflex sympathoinhibitory responses. The current experiments tested the possibility that circulating 3α-OH-DHP potentiates central GABAergic influences in the rostral ventrolateral medulla (RVLM). The unit activity of spontaneously active, sparsely projecting, and arterial pressure-sensitive neurons was recorded in the RVLM of urethan-anesthetized rats. Arterial pressure sensitivity of RVLM neurons was tested before (control) and 10 min after bolus injection (44 µl iv) of 3α-OH-DHP (1.12 µg/kg, n = 19) or vehicle (40% β-cyclodextrin, n = 8). Both threshold pressure and saturation pressure for inhibition of RVLM neurons were decreased after acute administration of a physiological dose of 3α-OH-DHP (1.12 µg/kg iv), which produces plasma concentrations similar to those seen during pregnancy (20–30 ng/ml), suggesting potentiated responsiveness to endogenously released GABA. Following suppression by 3α-OH-DHP, high doses of the inactive stereoisomer 3β-OH-DHP (112–224 µg/kg iv; n = 8) restored unit activity, presumably by displacing 3α-OH-DHP from the neurosteroid binding site on GABA<sub>A</sub> receptors.

γ-aminobutyric acid; baroreflex; progesterone

MATERIALS AND METHODS

Surgical preparation. Experiments were performed in 21 virgin female Sprague-Dawley rats (3–5 mo old; Harlan Sprague Dawley, Indianapolis, IN) weighing 225–270 g. Rats were anesthetized with intraperitoneal urethan (1.5 g/kg) supplemented (0.15 g/kg iv) as needed. A subcutaneous injection of dexamethasone (1.2 mg/kg) was also given to limit nervous tissue swelling. The trachea was cannulated, and the rat was artificially ventilated (CWE SAR830 ventilator) with O<sub>2</sub>-enriched room air. Body temperature was monitored and maintained at 37°C. An arterial catheter was placed into either the right carotid or right femoral artery to monitor arterial blood pressure, and three jugular catheters were anesthetized with intraperitoneal urethan (1.5 g/kg) and supplemented (0.15 g/kg iv) as needed. A subcutaneous injection of dexamethasone (1.2 mg/kg) was also given to limit nervous tissue swelling. The trachea was cannulated, and the rat was artificially ventilated (CWE SAR830 ventilator) with O<sub>2</sub>-enriched room air. Body temperature was monitored and maintained at 37°C. An arterial catheter was placed into either the right carotid or right femoral artery to monitor arterial blood pressure, and three jugular catheters were implanted for subsequent systemic drug administration. The rat was then placed in a stereotaxic apparatus, and an occipital craniotomy was performed. The occipital parietal dura were cut and folded laterally to expose the brain stem.

A laminectomy was performed to expose the spinal cord between C<sub>2</sub> and T<sub>2</sub>, and the spinal cord was stabilized on the same plane as the brain stem by means of a rigid clamp on the dorsal vertebral process of T<sub>2</sub>. The head was tilted forward until the calamus scriptorius was located 2.4 mm posterior to interaural zero (17). Tubocurarine (0.1 mg/kg iv) was administered to paralyze the rat, and a tungsten monopolar stimulating electrode (tip diameter 0.1 mm) was advanced into the dorsolateral funiculus on the left side of the spinal cord at the level of C<sub>2</sub> (immediately medial to dorsolateral sulcus and 0.3 mm ventral to dorsal surface). This region contains descending axonal projections from the RVLM to spinal preganglionic sympathetic neurons in the IML (32). A pressor response (20–40 mmHg) during brief electrical stimulation of the
spinal cord (5 mA, <1 ms, 5 Hz) verified the location of the electrode tip in the dorsolateral funiculus.

Drugs and solution. Urethan ethyl carbamate (99%) and phencyclidine (PE) were purchased from Sigma (St. Louis, MO). Tubocurarine chloride was obtained from Bristol Myers Squibb (Princeton, NJ). The urethan, tubocurarine, and PE were each diluted in isotonic saline. Dexamethasone sodium phosphate was purchased from Steris Laboratories (Phoenix, AZ). 2-Hydroxypropyl-β-cyclodextrin was purchased from Research Biochemicals International (Natwick, MA) and dissolved in a 50:50 solution of distilled water and isotonic saline to make 40% β-cyclodextrin. The progesterone metabolites 5α-pregnan-3α-ol-20-one (3α-OH-DHP) and 5α-pregnan-3β-ol-20-one (3β-OH-DHP), also obtained from Sigma, were dissolved in 40% β-cyclodextrin. Chicago sky blue 6B 80% was obtained from Aldrich (Milwaukee, WI), and neutral red was purchased from National Diagnostics (Highland Park, NJ).

Single unit recordings. Extracellular unit recording from cells in the RVLM was performed using glass microelectrodes (outer tip diameter =1 µm, resistance 1–2 MΩ) filled with 1% Chicago sky blue dye dissolved in 1 M NaCl. The electrode was advanced into the left side of the brain stem using a hydraulic microdrive (David Kopf). Spontaneously active neurons within anteroposterior coordinates of 0.5–0.8 mm rostral to the lateral funiculus, 0.7–2.2 mm lateral to midline, and 2.7–3.8 mm ventral to the dorsal surface were identified. The signal was amplified 10,000 times using two Grass (Quincy, MA) P15 preamplifiers and monitored on a loud-speaker as well as on a dual beam storage oscilloscope (R5031; Tektronics, Beaverton, OR). A rate meter/window discriminator (RAD-IIA, Winston Electronics) was used to determine the firing frequency of the unit. Unit activity (UA), heart rate, and MAP were monitored on a polygraph (79D, Grass Instruments) and stored on videotape (DR-886, Neuro Data Instrument) for later analysis using data acquisition software (RC Electronics, Computerscope).

Identification of spontaneously firing units as spinal projecting neurons involved in the regulation of cardiovascular function was determined by means of two different tests. First, spontaneous units were tested for antidromic spike production in response to electrical stimulation of the dorsolateral funiculus in the spinal cord (0.5 Hz, 5 mA, <1 ms). Neurons demonstrating a constant latency from the stimulus to the evoked spike and observation of collision with a spontaneous action potential suggested that the neuron projected to the spinal cord (Fig. 1). During antidromic activation of spontaneously active neurons, slight variability in latencies may occur due to the effect of different levels of membrane polarization on the delay between antidromic invasion of the spinal cord and the soma-dendritic region. Therefore, neurons with latency variation of <0.2 ms during repeated spinal cord stimuli were considered antidromically activated (20, 33). Second, the baroreflex sensitivity of the cell was tested. Slow ramp increases in MAP were elicited by graded intravenous injections (Razel infusion pump) of PE (9–300 µg/min) during the monitoring of the UA of the neuron. Arterial baroreceptors are rate sensitive and thus, for a given cell, care was taken to ensure that the rate of ramp increases in MAP was similar each time pressure sensitivity was tested. A progressive decrease in UA as MAP was elevated indicated arterial baroreflex sensitivity of the cell. Neurons that were both antidromically activated by dorsolateral funiculus stimulation and greatly inhibited by elevations in MAP were presumed to be presynaptic cardiovascular neurons of the RVLM (10) and were included in this study.

Experimental protocol. The relationship between MAP and UA was determined in RVLM neurons that met the above criteria during a gradual increase in MAP (control response). After MAP and UA returned to baseline values, a bolus intravenous injection of 3α-OH-DHP (1.12 µg/kg, n = 19) was administered. This dose was estimated to produce a maximum plasma concentration (~22 ng/ml) within the reported range of plasma concentrations seen during pregnancy (20–30 ng/ml) (28). Ten minutes after drug administration, the pressure sensitivity of the neuron was restated. The effect of vehicle (40% β-cyclodextrin iv) was evaluated in eight experiments using a similar protocol, except that an equivalent volume of 40% β-cyclodextrin (44 µl) instead of 3α-OH-DHP was administered.

Preliminary experiments evaluating the effects of a higher dose of 3α-OH-DHP were also performed. In seven cells that had received 1.12 µg/kg 3α-OH-DHP (iv), the effect of subsequent administration of a higher dose of 3α-OH-DHP (11.2 µg/kg iv) was also tested. The response of identified RVLM neurons to elevation of MAP was quantified by four parameters: MAP and UA values recorded at threshold and at saturation. After the experiments, taped data of MAP and instantaneous unit discharge were digitized (RC Electronics, Computerscope) and 5-ms averages were obtained (Microsoft Excel, Seattle, WA). Maximum
RESULTS

Identification and characterization of presympathetic RVLM pressure-sensitive neurons. For inclusion in the study, spontaneously firing neurons recorded in the RVLM were tested for antidromic activation that would indicate that these neurons were spinally projecting neurons. Figure 1 shows sequential oscilloscope traces of an RVLM unit that was antidromically activated by electrical stimulation of the dorsolateral funiculus in the spinal cord (0.5 Hz, 5 mA, 0.5 ms). A constant latency between the stimulus artifact and the evoked action potential (Fig. 1, A–C) and observation of collision of the stimulus with a spontaneous action potential (Fig. 1D) suggest that the neuron projected directly to the spinal cord. Baroreflex sensitivity of each neuron was also tested. Substantial inhibition of a neuron in response to increased MAP suggested that the neuron was part of the central baroreflex pathway.

Although many spontaneously firing neurons were recorded in the area of the RVLM, protocols were performed only on those neurons meeting the criteria described above. A total of 22 spontaneously active neurons were both antidromically activated from the dorsolateral funiculus in the spinal cord and inhibited by elevations in MAP. Elevated arterial pressure resulted in complete cessation of unit discharge in 15 neurons, and discharge was inhibited to 35.9 ± 9.0% of baseline in the remaining 7 neurons. These neurons were assumed likely to be presympathetic RVLM neurons involved in the baroreflex regulation of sympathetic outflow. Antidromic latencies ranging from 2 to 13 ms (mean 6.15 ± 0.8 ms) were observed in these neurons. Calculated conduction velocities, assuming a linear conduction pathway and an estimated distance of 2.5 cm between stimulus and recording site, ranged from 1.92 to 12.5 m/s (mean 5.9 ± 0.80 m/s). Baseline firing frequencies of identified neurons ranged from 3.2 to 32.4 pulses per second (pps). Of the 22 cells studied, pulse-synchronous activity was evident in 11 cells (Fig. 2). Although not stringently evaluated, several of the neurons appeared to also demonstrate a respiratory-like rhythm (n = 9).

Figure 3 shows a typical response of an RVLM neuron to a ramp increase in MAP. Parameters used to characterize responses are indicated on the figure. Effect of 3α-OH-DHP on baseline, threshold, and saturation parameters. The effects of vehicle and 3α-OH-DHP on presumed presympathetic, baroreflex-sensitive RVLM neurons were evaluated, and the results are summarized in Tables 1 and 2. Baseline values of MAP and UA measured immediately before the pressure ramps were not affected by either vehicle or 3α-OH-DHP (1.12 µg/kg iv, Table 1). Vehicle alone did not have an effect on threshold or saturation values in the eight neurons tested (Table 2). Responses to intravenous administration of 3α-OH-DHP (1.12 µg/kg iv) were determined in a total of 19 neurons. The maximum plasma concentration that could be achieved with this dose (≈22 ng/ml) was calculated to be within the range of concentrations seen in pregnancy (20–30 ng/ml) (28). Both the threshold MAP for inhibition of the unit and the saturation MAP were decreased by...
3α-OH-DHP (1.12 µg/kg iv), indicating an increased sensitivity of RVLM neurons to increases in arterial blood pressure. In five of these neurons, responses to intravenous administration of vehicle (44 µl 40% β-cyclodextrin) had been tested before 3α-OH-DHP. Statistical analysis of the data with and without these five neurons revealed the same significant differences, and therefore data from all 19 neurons are shown (Table 2 and Fig. 5).

Preliminary data evaluating the effect of subsequent administration of a higher dose of 3α-OH-DHP (11.2 µg/kg) were obtained in 7 of these 19 cells. Repeated-measures ANOVA on the subset of seven neurons exposed to both 1.12 µg/kg and 11.2 µg/kg 3α-OH-DHP revealed that subsequent administration of the higher dose of 3α-OH-DHP did not produce any further decrease in either threshold (117 ± 6.1 mmHg) or saturation MAP (152 ± 4.9 mmHg).

Effect of 3α-OH-DHP on recovery parameters. Recovery parameters for MAP and UA after the ramp increase in arterial pressure are summarized in Tables 3 and 4. Because of technical limitations or a prolonged time for recovery (>10 min), it was not possible to quantitate recovery parameters in all neurons. Recovery after PE-induced elevations in pressure was evaluated in 15 of 19 neurons exposed to 3α-OH-DHP (1.12 µg/kg). The t½ MAP (Table 3) and t½ UA (Table 4) were unaffected by vehicle or 3α-OH-DHP (1.12 µg/kg), indicating similar rates of recovery for both blood pressure and UA between control and treatment responses in these groups.

Recovery parameters were obtained for five of seven neurons exposed to the highest dose of 3α-OH-DHP (11.2 µg/kg iv) after administration of the physiological dose of 3α-OH-DHP (1.12 µg/kg iv). Repeated-measures ANOVA on control, 1.12, and 11.2 µg/kg 3α-OH-DHP values in these five cells revealed that t½ UA was increased [control, 84.2 ± 41.2; after 3α-OH-DHP (11.2 µg/kg), 136.5 ± 55.5 s] and MAP at t½ UA was less [control, 117.8 ± 7.8; after 3α-OH-DHP (11.2 µg/kg), 105.3 ± 4.0 mmHg] at the highest dose of 3α-OH-DHP. Therefore, in the presence of 11.2 µg/kg 3α-OH-DHP, recovery of UA was prolonged, and thus MAP was lower at t½ UA.

Effect of 3β-OH-DHP. Baseline values of MAP and UA obtained before (control) and 10 min after administration of 1.12 µg/kg 3α-OH-DHP (iv) were not different (Table 1). During the experiment, final MAP and UA values were also noted after the last PE-induced pressure ramp in the presence of 3α-OH-DHP.

The effect of administration of a high concentration of the inactive stereoisomer 3β-OH-DHP was evaluated

Table 1. Effects of treatments on baseline MAP and UA

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<th>Baseline MAP, mmHg</th>
<th>Baseline UA, pps</th>
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<tr>
<td>Control</td>
<td>93.07 ± 7.5</td>
<td>13.21 ± 2.7</td>
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<tr>
<td>Vehicle</td>
<td>92.29 ± 7.1</td>
<td>12.05 ± 2.8</td>
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<tr>
<td>Control</td>
<td>94.17 ± 2.8</td>
<td>13.64 ± 2.0</td>
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<tr>
<td>3α-OH-DHP (1.12 µg/kg)</td>
<td>93.11 ± 3.4</td>
<td>13.01 ± 2.3</td>
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Values are means ± SE. Neither baseline mean arterial pressure (MAP) nor baseline unit activity (UA) was significantly affected by iv administration of vehicle (40% β-cyclodextrin) or 3α-OH-dihydroprogesterone (3α-OH-DHP), pps, pulses per second.
in eight neurons that, at the time of the experiment, did not appear to fully recover after the final pressure ramp in the presence of 3α-OH-DHP (1.12 µg/kg, n = 5; 11.2 µg/kg, n = 3). MAP and UA for these eight neurons were compared immediately before and 1 min after bolus administration of high concentrations of 3β-OH-DHP (112–224 µg/kg). Despite a slight but significant increase in baseline MAP after administration of 3β-OH-DHP, a significant increase in UA was observed (Table 5). This is consistent with reversal of the effects of 3α-OH-DHP due to competition at the binding site by the inactive stereoisomer 3β-OH-DHP.

Histology. Post hoc histological examination of the recording sites verified that the neurons were distributed within an area previously described as the RVLM (Fig. 6) (3, 9, 10, 35).

DISCUSSION

The RVLM is an integral component in the central pathway for control of cardiovascular function and is considered to be the final site for tonic sympathoexcitatory drive to preganglionic sympathetic neurons in the spinal cord (10). Neurons in the RVLM receive and integrate cardiovascular input from both supramedullary and medullary nuclei (8). When arterial pressure increases, the medullary baroreflex pathway is activated. Increased discharge of afferent fibers from arterial baroreceptors results in excitation of neurons in the nucleus of the solitary tract (NTS), followed by excitation of neurons in the CVLM (8, 10). A monosynaptic projection from the CVLM to the RVLM (7, 24) inhibits tonically active neurons in the RVLM, which results in decreased discharge of preganglionic sympathetic neurons in the IML of the spinal cord (15). Inhibition of neurons in the RVLM by the CVLM is mediated by release of the amino acid neurotransmitter GABA (34). GABAergic influences represent the primary mechanism for arterial baroreflex inhibition of tonic sympathetic drive.

Earlier studies in our laboratory evaluating the effects of pregnancy on baroreflex function showed that pregnancy potentiates sympathoinhibitory and attenuated sympathoexcitatory baroreflex responses (6, 23). These effects are consistent with an increased GABAergic inhibition in the RVLM of pregnant animals. The mediator for these pregnancy-associated adaptations in control of sympathetic outflow is not known, but likely candidates are the ovarian hormones and/or their metabolites, which are elevated during pregnancy. It has been recently demonstrated that the primary metabolite of progesterone, 3α-OH-DHP, is the most potent endogenous positive modulator of CNS GABA_A receptor function (27, 28). Plasma concentrations of 3α-OH-DHP are elevated during pregnancy to levels that have been demonstrated to potentiate GABA-mediated inhibition (22, 28).

3α-OH-DHP belongs to a class of compounds known as neurosteroids whose primary action appears to be positive modulation of GABA_A receptor function. The mechanism of action is not thought to be genomic, because the effects are rapid (seconds to minutes) (22) and inhibition of protein synthesis does not alter the effect of neurosteroids (28). The neurosteroids bind to a

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<th>Table 3. Recovery parameters for MAP</th>
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<tr>
<td>Control 8</td>
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<td>Vehicle 8</td>
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<td>Control 15</td>
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<tr>
<td>3α-OH-DHP (1.12 µg/kg) 15</td>
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| Values are means ± SE. Neither vehicle nor 3α-OH-DHP at physiological dose affected half time for recovery of MAP (tᵢ) or UA at tᵢ, MAP. |

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<th>Table 4. Recovery parameters for UA</th>
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<td>MAP at tᵢ, UA</td>
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<td>Control 8</td>
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<td>Vehicle 8</td>
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<td>Control 15</td>
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<td>3α-OH-DHP (1.12 µg/kg) 15</td>
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Values are means ± SE. Neither vehicle nor 3α-OH-DHP (1.12 µg/kg) affected tᵢ, UA.

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<th>Table 5. Effect of 3β-OH-DHP on MAP and UA</th>
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Values are means ± SE. In a subset of neurons that were inhibited by 3β-OH-DHP, administration of the stereoisomer 3β-OH-DHP (maximum dose of 224 ng/kg) produced a significant increase in MAP and UA (*P < 0.05).
Characterization of presympathetic RVLM cardiovascular neurons. In the current study, spinally projecting spontaneously firing neurons in the RVLM that were inhibited by elevations in arterial pressure were presumed to be presympathetic neurons. Baseline UA of RVLM neurons included in this study also exhibited a wide range of firing frequencies, varying between 3.2 and 32.4 pps, which is consistent with the baseline firing rates reported by others (0.5-40 pps) (1, 2, 4, 9). Antidromic latencies between 2 and 13 ms (mean 6.15 ± 0.8 ms) and conduction velocities between 1.9 and 12.5 m/s were observed in this study. Conduction velocities ranging between 0.4 and 11 m/s have been reported (2, 4, 9, 16, 21, 26). The wide distribution of conduction velocities reported in the literature suggests involvement of both myelinated (>1 m/s) and unmyelinated (<1 m/s) fiber types. None of the neurons included in this study had conduction velocities <1 m/s, indicating that the axons of neurons in this study were probably myelinated. The mean conduction velocity of neurons included in this study (5.9 ± 0.8 m/s) is similar to that reported for presympathetic RVLM neurons by Granata and Kitai (5.5 ± 2.6 m/s) (9), Kanjhan et al. (4.9 ± 2.7 m/s) (16), and Lipski et al. (5.2 ± 2.3 m/s) (21). Mean conduction velocities reported by Morrison and Reis (26) are somewhat lower (3.1 ± 0.1 m/s). However, almost one-half the RVLM neurons characterized in that study were silent (49%) (26). Compared with conduction velocities of silent RVLM neurons, the conduction velocities of spontaneously active neurons tend to be higher (26). In our experiments, only spontaneously active RVLM neurons were studied, and thus we may have selected for neurons with myelinated axons and higher conduction velocities. Additionally, the use of relatively low-resistance electrodes (1-2 MΩ) in the current experiments would bias the recording toward larger cells with myelinated axons.

Although not used as a criterion in the positive identification of RVLM neurons, a correlation between neuronal firing pattern and cardiac cycle was observed in many units included in the study. As has been reported by others (4, 16), correlation between the cardiac cycle and unit discharge was evident primarily at elevated MAP, especially in those units exhibiting high baseline firing frequencies. Pulse synchrony was defined as a pattern of discharge that correlated with no more than one-half the cardiac cycle. With use of this criterion, evidence of pulse-synchronous discharge was observed in 11 of 22 neurons. The number of pulse-synchronous neurons may have been underestimated in this study. Because of the nature of the protocol, arterial pressure was not elevated for a prolonged period of time, which would have been necessary for definitive characterization of a neuron as pulse synchronous. It is likely that had enough data at high pressures been obtained, more neurons would have been characterized as pulse synchronous.

Although a direct correlation between UA and respiratory activity was not possible in this study because phrenic nerve activity was not recorded, a respiratory-like rhythm was noted in nine identified RVLM barore-

unique and stereospecific site on the GABA<sub>A</sub> receptor complex. Administered in 10- to 30-nM concentrations, neurosteroids are potent modulators of GABA<sub>A</sub> receptor function, prolonging the duration of chloride channel opening (22). Neurosteroids have been shown to produce an increased duration of inhibitory postsynaptic currents in hippocampal neurons (11). At higher concentrations (micromolar range), neurosteroids have been shown to directly open the chloride channel (22).

Previous studies in our laboratory evaluated the effect of acute administration of 3α-OH-DHP on baroreflex function in both anesthetized (14) and conscious rats (23). Acute administration of 3α-OH-DHP to virgin rats resulted in attenuated sympathoexcitatory responses and potentiated sympathoinhibitory responses. In other words, the acute response to exogenously administered 3α-OH-DHP in virgin rats was qualitatively similar to the effects of pregnancy. Although a CNS mechanism was implied by these previous studies, direct evidence was not provided. The purpose of this study was to determine if circulating levels of 3α-OH-DHP, administered in concentrations similar to those found in pregnancy, altered the sensitivity of sympathoexcitatory neurons in the RVLM to endogenously released GABA.

Fig. 6. Post hoc histological analysis of recording sites. Extracellular recording sites were marked with 1% Chicago sky blue dye in 1 M NaCl at end of each experiment by passing a 25-µA cathodal current through the electrode for 20 min. Serial sections through brain stem at level of RVLM show that neurons from which recordings were taken were indeed located in area of RVLM. Recording sites (21 neurons). Sol, nucleus of the solitary tract; Cu, cuneate nucleus; ECu, external cuneate nucleus; IO, inferior olive; Amb, ambiguous nucleus; Sp, spinal trigeminal tract.
flex-sensitive neurons. Of the nine neurons, five were additionally found to exhibit a pulse-synchronous pattern that was unmasked at high MAP. Recent reports have demonstrated an effect of central respiratory drive on the baroreflex at the level of the RVLM. Brown and Guyenet (4) demonstrated that spinally projecting barosensitive RVLM cells showing acute sensitivity to plasma CO₂ levels have a prominent respiratory-related rhythm. A direct correlation between phrenic nerve discharge and UA of baroreflex-sensitive RVLM neurons has been shown by Miyawaki et al. (25). However, Granata and Kitai (9) reported that antidromically activated RVLM neurons with respiratory-related activity demonstrated no baroreceptor-modulated activity. This apparent inconsistency may be due to the differences in recording sites between the studies. Compared with the study by Granata and Kitai (9), baroreflex-sensitive RVLM neurons with respiratory-related rhythm in the study by Miyawaki et al. (25) were located more dorsally in the RVLM. Although a respiratory modulation was evident in approximately one-third of the neurons in the current experiments, these neurons did not exhibit the characteristic on-off firing pattern of respiratory neurons and were greatly inhibited by elevated arterial pressure, suggesting that they were primarily involved in the arterial baroreflex (4, 16).

Post hoc histological verification of the recording sites for 21 neurons recorded in this study was obtained and revealed that the neurons were within the area described in the literature as the RVLM (10, 16, 25). In the current study, only 3 of the 21 neurons were located within 400 µm of the ventral surface. The majority of recorded neurons were located more dorsally (Fig. 6), and this may account for the relatively large number of neurons exhibiting respiratory-like rhythm. This distribution is consistent with reports by Brown and Guyenet (4) and Miyawaki et al. (25), demonstrating that a different number of neurons exhibiting respiratory-like rhythm. This distribution is consistent with reports by Brown and Guyenet (4) and Miyawaki et al. (25), demonstrating that sympathetic neurons 600-700 µm dorsal to the ventral surface of the medulla and 300-400 µm caudal to the caudal tip of the facial nucleus show a prominent respiratory rhythm.

The large number of dorsally located neurons may be a peculiarity of the experimental protocol of the current study. The recording electrode was advanced from the dorsal surface of the brain stem, and as soon as a spinally projecting pressure-sensitive neuron was isolated, the experiment was begun. Thus more ventral sites were frequently not explored.

Effect of 3α-OH-DHP on identified RVLM neurons. The majority of results in this study were obtained at a physiological dose of 3α-OH-DHP (1.12 µg/kg iv). This dose of 3α-OH-DHP was chosen to produce circulating levels of 3α-OH-DHP comparable to those seen during pregnancy. Maximal plasma concentrations achieved at this dose were calculated to be ~22 ng/ml, and normal plasma concentrations of 3α-OH-DHP during pregnancy are 20-30 ng/ml (28). At this dose, acute administration of 3α-OH-DHP produced a significant decrease in both threshold MAP and saturation MAP. This decrease in threshold and saturation pressures is consistent with an increased sensitivity of identified RVLM neurons to endogenously released GABA. The effects of 3α-OH-DHP were subtle (~10% change), as might be expected with a substance that modulates the response to an endogenous transmitter. Preliminary studies in which a higher dose of 3α-OH-DHP (11.2 µg/kg, n = 7) was administered revealed no further effect on threshold or saturation, indicating that near-maximal effects of 3α-OH-DHP are seen at physiologically relevant circulating levels.

Effect of 3α-OH-DHP on recovery parameters of RVLM neurons. 3α-OH-DHP was also found to have an effect on recovery after inhibition in response to elevations in pressure. Half time to recovery for MAP was not different between control and treatment for any of the groups (vehicle or 3α-OH-DHP). This indicates that once the PE stimulus was removed, MAP recovered at the same rate, thus eliminating the possibly confounding factor of different MAP recovery rates, which could affect recovery of the neuron. An effect of 3α-OH-DHP on recovery of UA was observed only in the presence of the highest dose of 3α-OH-DHP (11.2 µg/kg iv, n = 5) used in this study. At this dose, the t50 UA was significantly prolonged [control, 84.2 ± 41.2; after 3α-OH-DHP (11.2 µg/kg), 136.5 ± 55.5 s]. Also, as expected with a longer time period over which to recover, MAP at t50 UA was significantly lower [control, 117.8 ± 7.8; after 3α-OH-DHP (11.2 µg/kg), 105.3 ± 4.0 mmHg]. This effect on recovery is consistent with positive modulation of GABA receptors by 3α-OH-DHP. For a given level of GABA present, inhibition of neurons would be greater in the presence of 3α-OH-DHP compared with control. In the presence of 3α-OH-DHP, actual levels of endogenously released GABA would have to decrease further before UA could recover, and thus time to recovery would be prolonged.

Although plasma concentrations achieved after administration of the highest dose of 3α-OH-DHP (11.2 µg/kg iv) likely exceed plasma levels during pregnancy, the results are still potentially significant. The enzymes responsible for converting progesterone to neuroactive metabolites are located both peripherally and within the CNS. Both circulating and centrally synthesized progesterone are converted to 3α-OH-DHP in the brain, and CNS concentrations of 3α-OH-DHP may exceed plasma concentrations by 100-fold (31). Thus acute intravenous administration of the higher dose of 3α-OH-DHP in the current experiments would result in acute exposure of the brain to concentrations that might well be within the physiologically relevant range for the CNS.

Effect of 3β-OH-DHP on RVLM neurons. Currently, specific antagonists for the neurosteroid binding site are not available. However, high concentrations of the inactive stereoisomer 3β-OH-DHP have been shown to compete with 3α-OH-DHP for binding sites and thereby reverse the positive modulation of GABA receptors by 3α-OH-DHP (30). In the current experiments, any modulatory effect of 3α-OH-DHP on baseline firing rate would be most evident after a manipulation whereby endogenous GABA is elevated (i.e., after increased
MAP). In 8 of 19 RVLM neurons, incomplete recovery of both MAP and UA was observed after the final pressure ramp in the presence of 3α-OH-DHP (11.2 µg/kg, n = 3; 1.12 µg/kg, n = 5), suggesting an inhibitory effect. In these neurons, the effect of the inactive stereoisomer 3β-OH-DHP was evaluated. The relatively high dose of 3β-OH-DHP (112–224 µg/kg) used in this study was chosen in an effort to produce maximal competition at the neurosteroid binding site on GABA_A receptors. Baseline levels of MAP increased slightly with administration of 3β-OH-DHP. However, despite the slight increase in pressure, 3β-OH-DHP produced a significant increase in UA within 1 min of administration, indicating reversal of the inhibitory effect of 3α-OH-DHP. Additionally, the rapid effect of 3β-OH-DHP further suggests that the mechanism of action of the neuroactive metabolite of progesterone, 3α-OH-DHP, was through a stereospecific nongenomic action at a unique binding site on the GABA_A receptor complex.

Although the results of this study show that 3α-OH-DHP, administered to achieve plasma concentrations similar to those seen in pregnancy, has an effect on neurons in the RVLM, it should be recognized that the actual site of action of 3α-OH-DHP remains uncertain. 3α-OH-DHP is a highly lipid-soluble molecule and therefore has access to all CNS sites after intravenous administration. GABAergic influences have been demonstrated in other medullary nuclei in the baroreflex pathway, including the NTS and the CVLM (8). However, potentiation of GABAergic responses in the NTS or the CVLM might be expected to produce sympathoexcitation and attenuation of baroreflex sympathoinhibition (10), an opposite effect from that observed in both this and previous baroreflex studies (14, 17, 23). As the final site for sympathoexcitative influences, the RVLM is the most likely site in the medullary baroreflex pathway where an increase in GABAergic influences would produce a potentiation of sympathoinhibition.

One potential mechanism for the preferential effect of 3α-OH-DHP in the RVLM would be a greater affinity for 3α-OH-DHP by RVLM neurons compared with other regions involved in the central baroreflex pathway. Affinity of 3α-OH-DHP for the GABA_A receptor is dependent on the subunit composition of the receptor. Studies have demonstrated that although the β-subunit of the GABA_A receptor complex has no effect on modulation of GABA-induced chloride current, different α- and γ-subunit isoforms (12, 19) may significantly affect the efficacy of 3α-OH-DHP to modulate GABA_A receptor binding and function (28). Although it has not been determined in the medulla, heterogeneity of GABA_A receptors in other areas of the CNS has been proposed to account for regional differences in neurosteroid responsiveness (28). Thus it is possible that GABA_A receptors are distributed such that more receptors in the RVLM contain the appropriate subunits to maximize modulation of the GABA_A receptor complex by 3α-OH-DHP.

Lastly, although this study demonstrated an effect of intravenous 3α-OH-DHP on arterial pressure sensitivity of RVLM neurons, the CNS site of action for the effects of 3α-OH-DHP on control of sympathetic outflow may not necessarily be restricted to the RVLM. The RVLM receives tonic excitatory drive from several supramedullary structures (8, 10), and it is possible that potentiation of GABAergic inhibition at one of these sites could have contributed to the results of the current experiments.

Perspectives

The current study demonstrated that acute increases in circulating levels of the neuroactive metabolite of progesterone, 3α-OH-DHP, result in potentiation of baroreflex inhibition of brain stem RVLM neurons. The fact that near-maximal effects were observed after a dose calculated to produce plasma concentrations within the range seen during pregnancy was administered suggests that these results may be physiologically relevant. Thus variations in levels of ovarian hormones and their metabolites, as occur during the estrus cycle and during pregnancy, may affect CNS regulation of sympathetic outflow and cardiovascular function. Although acute administration of the progesterone metabolite to virgin female animals produced effects qualitatively similar to the effects of pregnancy, the effects of long-term exposure to elevated levels of 3α-OH-DHP, as would occur in pregnancy, remain to be evaluated. In addition, preliminary experiments in which baroreflex control of efferent sympathetic nerve activity has been evaluated in male (13) and ovariectomized female rats (18) suggest that prior exposure to ovarian hormones is necessary for the acute effects of 3α-OH-DHP to be fully evident. Thus it is likely that modulation of sympathetic outflow by ovarian hormones and their metabolites is the result of an interaction between genomic and nongenomic actions within the CNS.

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REFERENCES


Cauterization of the lateral hypothalamic area prevents the high blood pressure response to attenuation of baroreflex control of sympathetic outflow. Am. J. Physiol. 266 (Heart Circ. Physiol. 35): H1080–H1086, 1994.