Activation of serotonin 1A (5-HT$_{1A}$) receptors has been shown to have diverse effects on respiration. The purpose of this study was to determine changes in respiratory motor pattern of phrenic nerve activity and respiratory rhythm after systemic application of specific 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-di-n-propylamino-tetralin (8-OH-DPAT). We hypothesized that systemic application of specific 5-HT$_{1A}$ receptor agonist 8-OH-DPAT in spontaneously breathing anesthetized rats will enhance phrenic motor output and phrenic respiratory rate. The study was performed in spontaneously breathing urethane anesthetized rats. Intravenous application of 8-OH-DPAT produced dose dependent increase in the amplitude of integrated phrenic nerve activity and disturbances in respiratory rhythm. Stimulating effect of 8-OH-DPAT on phrenic nerve activity was abolished by intravenous application of the selective 5-HT$_{1A}$ receptor antagonist WAY, N-(2-(4,2-methoxyphenyl)-1-piperazinyl)ethyl)-N-2-pyridinyl-cyclohexane-carboxamide maleate (WAY-100635). These results show that stimulation of 5-HT$_{1A}$ receptors by intravenous application of 8-OH-DPAT enhances phrenic nerve activity in spontaneously breathing rats.

Key words: phrenic nerve recordings, serotonin, respiration, rats

INTRODUCTION

Serotonin (5-HT) has been known as an important modulator of respiratory rhythm. However, studies of 5-HT effects on respiratory control have shown
inconsistent results. The different results could be related to differences in species, doses administered, or in the methods used. Underlying mechanisms are likely complex but some of the observed variability in effects of serotonin may be explained by expression of different subtypes of 5-HT receptors on respiratory neurons. Activation of 5-HT_{1A} receptors had positive effect on disturbed respiratory function as shown in several different approaches where 8-OH-DPAT was used in restoring normal breathing (1 - 4). The central respiratory behavior of anesthetized animals is generally described by reference to phrenic nerve activity. 8-OH-DPAT given intravenously altered the respiratory rhythm in cats and led to phrenic apnea in higher doses (5). However, there is a little information on the influence of 5-HT_{1A} receptor activation on the pattern and rhythm related changes of the phrenic nerve activity in rats. Therefore, the present study was undertaken to examine the effects of intravenous application of specific 5-HT_{1A} receptor agonist 8-OH-DPAT in spontaneously breathing anaesthetized rats on rhythm and pattern related changes in the phrenic motor output.

METHODS

The protocol for this study was approved by the Ethical Committee for Biomedical Research of the University of Split School of Medicine, Split, Croatia. All experiments were carried out in accordance with the National Research Council's guide for the care and use of laboratory animals.

**General Procedures.**

Experiments were performed on 20 adult male Sprague-Dawley rats weighing 280-330 g. Anesthesia was performed with intraperitoneal injection of 20% solution of urethane in 0.9% saline (1.2 g/kg; supplemental dose 0.2 g/kg). The adequacy of anesthesia was assessed by absence of a withdrawal reflex after noxious paw pinch. The femoral vein and artery were cannulated for intravenous drug delivery, blood pressure monitoring, and sampling of arterial blood. Blood samples were taken at intervals, and arterial blood gasses were maintained within physiological limits by infusion of bicarbonate solution. The trachea was cannulated through midline incision. All animals were vagotomized bilaterally. End-tidal CO$_2$ concentration was continuously monitored with a GEMINI respiratory gas analyzer (CWE Inc., USA) and maintained within physiological limits. Rectal temperature was monitored by digital thermometer and maintained between 37 and 38.5°C by means of external heating pad (FST, Germany).

The rats were placed in a prone position in a stereotaxic instrument (Lab Standard, Stoelting, USA). The right phrenic nerve was dissected using dorsal approach at the level of C5 nerve rootlet, mounted on bipolar silver wire electrodes and covered with silicone gel to prevent from drying. Phrenic nerve activity was amplified, filtered (band-pass 300 Hz-10 kHz) and rectified; the moving time average of phrenic nerve activity was obtained using MA-1000 Moving Averager, System 1000 Modular Instrumentation (CWE Inc., USA) with a 50-ms time constant.

**Experimental protocols.**

Two experimental protocols using separate groups of animals were performed. In the first protocol (n = 15) we examined the effect of intravenous application of 8-OH-DPAT on phrenic
nerve activity. 8-OH-DPAT (Sigma Aldrich Chemie GmbH, Germany) was dissolved in 0.9% saline and administered as a bolus with a time duration of 10 to 15 seconds, in three different dosing regimens, 25 µg/kg (n = 5), 50 µg/kg (n = 5) and 100 µg/kg (n = 5).

In the second protocol (n = 5) 5-HT$_{1A}$ receptor antagonist WAY-100635 (Sigma Aldrich Chemie GmbH, Germany) was dissolved in 0.9% saline and administered in a dose of 0.3 mg/kg either 1 min prior to the application of 8-OH-DPAT or 1 min after the application of 8-OH-DPAT.

Prior to each intravenous injection of either agonist (8-OH-DPAT) or antagonist (WAY-100635) of 5-HT$_{1A}$ receptors, the same volume of 0.9% saline was injected.

**Statistical analysis.**

The response to injections of 8-OH-DPAT was analyzed on the phrenic motor output in terms of inspiratory duration (T$_I$), expiratory duration (T$_E$), respiratory cycle duration (T$_{TOT}$), and peak amplitude of phrenic nerve discharge. Nerve activity and blood pressure were averaged over a 10 seconds period and expressed as percentage changes from the pre-injection control. Baseline values for all of the variables were determined by averaging these values 10 seconds prior to intravenous

---

![Fig. 1. Responses of arterial blood pressure (BP), integrated phrenic nerve activity (IPNA), and averaged phrenic nerve activity (RPNA) to intravenous injection of (A) 25 µg/kg of 8-OH-DPAT, (B) 50 µg/kg of 8-OH-DPAT, and (C) 100 µg/kg of 8-OH-DPAT.](image-url)
application of drugs. Amplitude of integrated phrenic nerve activity was normalized to pre-injection baseline activity, which was set at 100% in each animal.

All the values are reported as means ± S.E. Analysis of variance was used for comparison between the groups followed by Tukey HSD post hoc test. Statistical significance was set at \( p<0.05 \).

**RESULTS**

The results indicate that intravenous administration of 5-HT\(_{1A}\) receptor agonist 8-OH-DPAT enhances phrenic nerve activity in spontaneously breathing anesthetized rats. These effects were abolished by application of selective 5-HT\(_{1A}\) receptor antagonist, WAY-100635.

*Table 1.* The relative changes of the amplitude of phrenic nerve, inspiratory duration (\( T_I \)), expiratory duration (\( T_E \)), and respiratory cycle duration (\( T_{tot} \)) depending on the dose of 8-OH-DPAT administered.

<table>
<thead>
<tr>
<th>Dose( \mu g/kg )</th>
<th>N</th>
<th>Increase in amplitude of PNA (%)</th>
<th>( T_I ) (relative change, %)</th>
<th>( T_E ) (relative change, %)</th>
<th>( T_{tot} ) (relative change, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>5</td>
<td>26±1.5</td>
<td>-2.3±2.2</td>
<td>9.4±2.4</td>
<td>4.5±1.4</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>24±2.9</td>
<td>12.4±2.7*</td>
<td>-1.0±4.4</td>
<td>1.3±2.9</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
<td>73.1±6.3**</td>
<td>13.7±3.3*</td>
<td>-11.1±3.1*</td>
<td>-2.0±2.6</td>
</tr>
</tbody>
</table>

†Dose of 8-OH-DPAT administered intravenously.
*Significantly different (\( p<0.05 \)) from effects elicited by intravenous injection of 8-OH-DPAT in the dose of 25 \( \mu g/kg \).
** Significantly different (\( p<0.05 \)) from effects elicited by intravenous injection of 8-OH-DPAT in the dose of 25 \( \mu g/kg \) and 50 \( \mu g/kg \). All values are means ± S.E. of relative changes.

*Fig. 2.* Percentage changes in phrenic nerve activity after intravenous injection of 25, 50, and 100 \( \mu g/kg \) of 8-OH-DPAT. Variability is indicated by standard error bars; *denotes significant difference in the changes of phrenic nerve activity compared to doses of 25 and 50 \( \mu g/kg \), \( p<0.05 \).
Administration of 8-OH-DPAT produced significant increases in the amplitude of the phrenic nerve activity (Fig. 1). The effects elicited by 8-OH-DPAT injections on respiratory rhythm are presented in Table 1. Increase in the amplitude of phrenic nerve was significantly higher for the dose of 100 µg/kg compared to 50 µg/kg, and 25 µg/kg of 8-OH-DPAT (Fig. 2). Onset of changes in the phrenic nerve activity started at 5 - 11 seconds after i.v. application of 8-OH-DPAT. Phrenic nerve activity remained enhanced for the period of 20-40 min following the injection of 25 µg/kg of 8-OH-DPAT, and for 120-180 min following the injections of 50 and 100 µg/kg of 8-OH-DPAT and than slowly returned to the baseline (Fig. 3). There was no significant difference in the baseline T_i, T_e and TTOT (Table 2). Baseline blood pressure was not different between the three groups (124 ± 8.9 mmHg, 110 ± 7.2 mmHg, and 106 ± 8.8 mmHg), nor the magnitude of the hypotension which accompanied each dose of 8-OH-DPAT administered.

The effects of WAY-100635 were studied in five spontaneously breathing animals. WAY-100635 administered intravenously did not produce changes in the phrenic nerve activity, respiratory frequency, and blood pressure. 8-OH-DPAT (50 µg/kg or 100 µg/kg) failed to alter phrenic nerve activity after WAY-100635. In one animal, a single dose of WAY-100635 (0.3 mg/kg) was

![Fig. 3. Response of arterial blood pressure (BP), integrated phrenic nerve activity (IPNA), and averaged phrenic nerve activity (RPNA) to intravenous injection of 50 µg/kg of 8-OH-DPAT, and return to the baseline after 120 min.](image)

<table>
<thead>
<tr>
<th>Dose† µg/kg</th>
<th>N</th>
<th>RF (breath/min)</th>
<th>T_i (s)</th>
<th>T_e (s)</th>
<th>TTOT (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>5</td>
<td>49.8±3.45</td>
<td>0.38±0.02</td>
<td>0.8±0.11</td>
<td>1.2±0.1</td>
</tr>
<tr>
<td>50</td>
<td>5</td>
<td>45.6±3.6</td>
<td>0.39±0.04</td>
<td>0.97±0.11</td>
<td>1.36±0.11</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
<td>50.1±1.75</td>
<td>0.44±0.04</td>
<td>0.77±0.06</td>
<td>1.21±0.03</td>
</tr>
</tbody>
</table>

†Dose of 8-OH-DPAT administered intravenously.
All values are means ± S.E. of absolute changes.
administered 1 min after the application of 8-OH-DPAT (100 µg/kg) which had enhanced the phrenic nerve activity (Fig. 4). WAY-100635 antagonized the effects of 8-OH-DPAT (Fig. 4) and shortened the duration of 8-OH-DPAT effects on phrenic nerve activity (Fig. 4B).

Intravenous application of 0.9% saline, had no effect on respiratory rhythm and respiratory motor pattern of phrenic nerve activity.

DISCUSSION

This study demonstrates that intravenous administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT enhances phrenic nerve activity in spontaneously breathing rats. In the first part of this study it was consistently observed that stimulating effects depended on the dose administered. In the second part of this study, 8-OH-DPAT induced facilitatory effects that were completely antagonized by intravenous injection of WAY-100635.

Previous studies (6, 7) provided evidence for the involvement of 5-HT in respiratory control. The effects of serotonin on respiration are complex and at least partially can be explained by stimulation of different 5-HT receptor subtypes.
Agonists of 5-HT$_{1A}$ receptors were shown to be useful in counteracting respiratory depression (1, 2, 8). 8-OH-DPAT reversed morphine and dizocilpine induced apnea in anesthetized rats (1), and restored normal breathing in spinal cord injured rats (2). The unpublished observation from our laboratory showed that morphine induced cessation of phrenic nerve activity was recovered by intravenous application of 8-OH-DPAT, and this effect was abolished by intravenous application of 5-HT$_{1A}$ receptors antagonist WAY-100635. This finding strengthen the evidence for the stimulating effects of 8-OH-DPAT on the phrenic nerve activity in both morphine disturbed and normal breathing.

Different ways of agonist administration may play an important role in opposite effects observed on respiration (5, 9). Intravenous administration of 8-OH-DPAT can influence the neurons in the phrenic motoneuron nucleus (PMN) directly, or indirectly through the neurons in the ventral respiratory group (VRG) that project to the PMN (10, 11). A major source of serotonin in the brain is raphe nuclei. Raphe nuclei was shown to send efferent projections to the VRG (10), and to the PMN (12). Serotonin-containing neurons originating from the raphe magnus, raphe obscurus and raphe pallidus project to the rostral ventral respiratory group (13-15). In more rostrally located raphe nuclei non-serotonergic neurons, presuming GABA-ergic (12), were also found to project to the rostral ventral respiratory group (14). These projections may represent the neuroanatomic substrate for their involvement in the respiratory regulation. A possible explanation for the diverse effects of 5-HT$_{1A}$ agonists on respiratory function can be found in two opposite suggestions regarding the site and underlying mechanism. Excitatory effects might be explained by activation of 5-HT$_{1A}$ autoreceptors that lead to inhibition of central serotonergic system (1). In contrast, inhibitory effects might be explained by activation of 5-HT$_{1A}$ postsynaptic receptors (5).

Since respiratory rhythm generation features both excitatory and inhibitory neurotransmitters, activation of 5-HT$_{1A}$ receptors may influence final outcome in complex regulation of primary respiratory network. It is not known whether there are differences regarding mechanism and/or site of action of 5-HT$_{1A}$ receptor agonists depending on systemic or central administration of these drugs. Systemic administration would have activated broadly distributed 5-HT receptors including respiratory areas in the brainstem. In addition, systemic administration of nonselective 5-HT receptor antagonist methysergide inhibited facilitatory effect produced by central stimulation of the raphe pallidus (12). Systemic administration of specific 5-HT$_{1A}$ receptor agonist has beneficial effects on respiratory disturbances (2, 8). However, no clear picture emerges as to whether the systemic activation of 5-HT$_{1A}$ receptors enhances or diminishes respiration in anesthetizes animals. We became interested in this problem because of the contradictory findings that were obtained in different studies with no change (1) or decrease (5) in respiratory output following the systemic administration of 5-HT$_{1A}$ receptors agonist. Systemic administration of 8-OH-DPAT in our study
produced a clear and prompt excitatory response of the phrenic nerve activity. Additionally, excitatory response depended on the dose of 8-OH-DPAT administered. Therefore, prior to central stimulation, we focused on the changes in the phrenic nerve activity following systemic application of 5-HT$_{1A}$ receptors agonist. Further studies from our group will focus on the central mechanism(s) and site of action of specific 5-HT receptors in the breathing control.

The limitation of the current study is inability to support the mechanism and site of action of 8-OH-DPAT on phrenic nerve discharge, but the rationale given was supported by the results from the previous studies (12). Additionally, it should be noted that some anesthetics influence the resting function of the respiratory and cardiovascular system. However, the urethane in anesthetic doses has minimal or no effect on resting respiratory function (16). Sapru and Krieger reported that urethane does not effect respiratory rate and tidal volume in decerebrated rats (17). Additionally, interaction of serotonergic agents and hypoglossal phasic activity was not eliminated under urethane anesthesia (18). Therefore, when administered by the proper route and at appropriate doses, this anesthetic is suitable for a variety of physiopharmacological studies at respiratory and cardiovascular system level.

In summary, our data clearly suggest that systemic administration of 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT stimulates phrenic nerve activity in spontaneously breathing anesthetized rats.

Acknowledgments: The authors wish to thank Caron Dean, Eckehard Stuth, and Zoran Valic for scientific revision of the manuscript, and to Jelena Baricevic for her technical assistance.

This work was supported by the Croatian Ministry of Science, Education and Sport Grants 0216003 and 0216015.

REFERENCES


Received: August 2, 2007
Accepted: January 22, 2008

Author’s address: Zoran Dogas, Department of Neuroscience, University of Split School of Medicine, Soltanska 2, 21 000 Split, Croatia; tel: ++ 385 21 557 934 or ++ 385 21 557 905, fax: ++ 385 21 465 304; e-mail: zdogas@bsb.mefst.hr