This study tested the role of inhibitory neurotransmission in the glutaminergic control of short-term depression (STD) of the inspiratory activity initiated by sustained stimulation of the vagus nerve in anesthetized and vagotomized cats. STD, calculated from the integrated phrenic nerve signal, lasted longer when glutaminergic neurotransmission was inhibited by ketamine, a NMDA receptor antagonist. Application of picrotoxin, a GABA receptor antagonist, reversed the effect of ketamine and shortened the STD duration below that present in the control condition. The results showed that alternation of the neural excitability by antagonists of excitatory and inhibitory neurotransmission modulates the STD of inspiratory activity, evoked by vagal stimulation. The STD depends on the state of neural excitability and is easier accomplished when the excitability is on the high side.

**Key words:** GABA, inspiratory activity, NMDA, short-term depression

**INTRODUCTION**

Integrative processes in neural control of breathing were described for many afferent inputs to the respiratory controller. They originate from central (pontine, mesencephalic) and peripheral (vagal, carotid sinus nerve) sources (1, 2). Both facilitation and inhibition are induced depending on the afferent input stimulated and the type of stimulation. Phasic input from the vagus nerve reflexly regulates the duration of respiratory phases and the resulting intensity of inspiratory activity. Stimulation of the vagus nerve applied in a given respiratory phase (1) or sustained during a longer time (3) causes reversible changes, with a different
time constant, in breathing frequency and the duration of respiratory phases. The features of habituation and desensitization and other processes activated during vagal stimulation have been studied in rats (3). The post-stimulatory effects are described mostly for respiratory timing. Blockade of the NMDA neurotransmission by dizocilpine modulates the time-dependent respiratory responses to vagal stimulation (3). However, this study has not discerned the post-stimulatory short-term depression (STD) of inspiration in detail (3).

Ketamine, an antagonist of NMDA receptors, changes the respiratory pattern eliciting an increase of inspiratory duration and a decrease of inspiratory amplitude. The GABA receptors sensitive to picrotoxin appear to play a role in shaping the ketamine-induced pattern of breathing (4). The current study focused on the post-stimulatory time-related effects of tonic vagal stimulation on the intensity of inspiratory activity. Specifically, we investigated the role of the excitatory and inhibitory neurotransmissions in the STD of inspiratory activity during tonic vagal stimulation in the cat.

MATERIAL AND METHODS

A local Ethics Committee approved the experimental protocol of this study. Six adult cats anaesthetized with mixture of α-chloralose (40 mg/kg) and urethane (1 g/kg), ip, were used for the experiments. The animals were tracheostomized, paralyzed with pipecuronium bromide (Arduan, Gedeon-Richter, Budapest, Hungary), and mechanically ventilated. Femoral artery and vein were catheterized for monitoring the arterial blood pressure and for injections of chemical agents, respectively. Both vagus nerves were cut in the mid-cervical region. Left C5 phrenic nerve root was cut distally and placed on a bipolar silver electrode. Arterial blood pressure was measured with MCK 4011S (Femed, Zabrze, Poland) and the arterial blood gas content and pH with Blood Gas Assembly (AVL Compact 2, Graz, Austria). End-tidal concentration of CO₂ was continuously controlled (Respiratory Gases Meter Respina IH26 - NEC San-ei Instruments, Tokyo, Japan) and kept within normal limits. Rectal temperature was maintained at 37-38°C.

Action potentials of the phrenic nerve were amplified and filtered (0.05-5.0 kHz) with a NeuroLog System (Digitimer, Welwyn Garden, UK) and integrated with a time constant of 70 ms. Raw and integrated phrenic nerve signals, arterial blood pressure, end-tidal CO₂, and a stimulus marker were sampled at 2.5 kHz rate by an Adcjul Acquisition System (Warsaw, Poland) and stored for off-line analysis.

For vagal stimulation, the central stump of the left vagus nerve, contralateral to the phrenic nerve activity recorded, was placed on silver stimulating electrodes. Electrical stimulation consisted of a 20 s train of stimuli of 0.5 ms duration and 100 Hz frequency. The intensity of stimulation was three times above the threshold for any evident change in respiratory pattern. Stimulation started in the expiratory phase.

A non-competitive NMDA receptor channel antagonist, ketamine (Ketalar, Gedeon-Richter, Budapest, Hungary) at a dose of 0.5 mg/kg, and a non-competitive GABA₄ antagonist, picrotoxin (Sigma-Aldrich, Poznań, Poland) at a dose of 0.2 mg/kg, were administered as a bolus injection via femoral vein. Agents were dissolved in 0.9% NaCl.

The experiment began when the respiratory pattern was stabilized after the completion of all experimental preparations. The intensity of vagal stimulation was established for each experiment and was the same at all stages of the experiment. Vagal stimulation with the chosen intensity was
applied during the control condition and 15 min following ketamine and picrotoxin injections. The dose of ketamine was chosen as the lowest that evokes an apneustic type prolongation of inspiration in the cat. Time elapsed between each vagal stimulation was at least 20 min.

The respiratory effects of vagal stimulation were estimated from the integrated phrenic neurogram in the unilaterally phrenectomized animals. Peak height of the phrenic amplitude was taken as the neural tidal volume, the duration of a phrenic burst from its onset to the peak and from the peak to the next onset was taken as the inspiratory and expiratory time, respectively. Baseline respiratory variables before vagal stimulation were averaged over 5 consecutive phrenic bursts. Instantaneous frequency was calculated from the first respiratory cycle after stimulation. Variables during and after stimulation were calculated for each breath as long as the response lasted. Duration of the STD was estimated as the period of time and/or the number of breaths necessary for restoration of phrenic amplitude to the level present prior stimulation. Data were calculated as a percent of control before and after each pharmacological treatment and are given as mean ±SE. Differences in phrenic amplitude among the control, ketamine, and picrotoxin settings, evoked by vagal stimulation, were assessed with one-way analysis of variance. Statistical significance was considered at P<0.05.

RESULTS

Firstly, we examined the effects on phrenic nerve activity, taken as neural respiratory output, of a 20 s electrical stimulation of the central end of the cut vagus nerve. A representative original recording demonstrating the respiratory effects of such stimulation is shown in Fig. 1. The response consisted of apnea terminated by the end of stimulation in two cases. In another four cases the effect of stimulation was dynamic, consisting of a prolonged expiratory phase followed by a shortened inspiratory phase and reduced inspiratory amplitude. As stimulation continued the immediate response habituated; the expiratory duration shortened and phrenic burst amplitude increased, but remained lower than that before stimulation.

At the end of stimulation the instantaneous frequency of phrenic bursts returned to the control level in the first or second respiratory cycle with only a

![Fig. 1](Image)

*Fig. 1*. Original recording of the effect of vagal stimulation (Stim) on integrated phrenic nerve activity (IPhr) and blood pressure (BP). During stimulation the initial apnea changed to rhythmic, low amplitude activity.
slight sign of post-inhibitory rebound. Amplitude of the phrenic activity 
recovered slowly, exhibiting the STD (Fig. 2) that lasted for 39.1 ±9.9 s.

Injection of ketamine at a dose of 0.5 mg/kg evoked typical changes in 
respiratory activity (4). Apneustic pattern of breathing invariably appeared that 
consisted of increased inspiratory time, almost unchanged expiratory time, and 
decreased peak of phrenic amplitude. Here, stimulation of the vagus nerve with the 
same intensity as that in the control condition caused a comparable inhibition of 
inspiratory activity and also some habituation of the response to stimuli, although 
it varied among animals. The amplitude of the first post-stimulatory phrenic burst 
was significantly lower in comparison with that in the control and picrotoxin 
(P<0.05). Under ketamine the post-stimulatory respiratory pattern became more irregular than 
before stimulation; prolonged inspirations alternating with short ones.

Picrotoxin administered 20 min after ketamine reversed the respiratory effects 
of ketamine by increasing the frequency and amplitude of phrenic bursts. During 
vagal stimulation both inhibition of inspiratory activity and subsequent 
habituation were comparable with that during control and ketamine. However, 
picrotoxin decreased significantly the duration of the post-stimulatory STD of

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**Table 1.** Mean respiratory frequency before vagal stimulation and mean instantaneous frequency 
of the first post-stimulatory breath.

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Baseline</th>
<th>Post-stimulation</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>15.9 ±1.3</td>
<td>16.4 ±3.6</td>
</tr>
<tr>
<td>Ketamine</td>
<td>10.3 ±1.4</td>
<td>11.1 ±2.7</td>
</tr>
<tr>
<td>Picrotoxin</td>
<td>16.2 ±2.0</td>
<td>20.5 ±4.1*</td>
</tr>
</tbody>
</table>

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Fig. 2. Relative changes in integrated phrenic nerve amplitude in consecutive breaths after cessation of the vagus nerve stimulation in the control condition and following ketamine and picrotoxin administration. The amplitude of post-stimulatory breaths was normalized to the averaged phrenic amplitude (C) immediately preceding stimulation. Values are means ±SE. Asterisk denotes a significant difference of the first post-stimulatory amplitude compared with that in the control and picrotoxin (P<0.05).
phrenic amplitude (to 32.2 ±9.8 s) that became comparable to that after ketamine, but shorter than in the control.

Instantaneous frequency of inspiratory bursts after vagal stimulation exhibited a post-inhibitory rebound (PIR) under picrotoxin, which was rather weak after the preceding vagal stimulations (Table 1). In all conditions studied, the arterial blood pressure during stimulation of the vagus nerve remained unchanged or slightly decreased.

DISCUSSION

The present study demonstrates that tonic vagal stimulation elicited post-stimulatory changes in peak phrenic activity that corresponded to STD, one of the forms of neural adaptation. The STD of inspiratory activity was sensitive to NMDA and GABA receptor antagonists.

Inspiratory activity running down to phrenic motoneurons is formed by excitatory processes relayed by NMDA and non-NMDA receptors at medullary inspiratory neurons (5). The NMDA receptors carry tonic excitatory drive to inspiratory neurons during the silent period of neuronal activity (6). Activation of NMDA and non-NMDA receptors (7) is also involved in the central and vagal mechanisms of inspiratory termination, respectively. Pharmacological blockade of NMDA receptors by ketamine influences all of the above mechanisms and elicits inhibition of both inspiratory activity and inspiratory termination. Since the time constant of inspiratory STD becomes longer when the tonic drive is reduced by NMDA antagonism (8), the amount of the excitatory drive is likely responsible for the duration of inspiratory STD.

A short-term adjustment of respiratory output following removal of the vagal inhibitory input may also result from increased accumulation of an inhibitory neurotransmitter evoked by sustained stimulation. A previous study (4) has shown that the respiratory effects of ketamine are modulated by GABA<sub>A</sub> receptors, specifically by receptors sensitive to picrotoxin, a non-competitive GABA<sub>A</sub> antagonist. In the current study picrotoxin restored the amplitude of inspiratory activity depressed by ketamine and reduced an apneustic prolongation of the inspiratory phase. Picrotoxin eliminated the ketamine-induced prolongation of STD and shortened it even more to the level comparable with the control condition. This means that picrotoxin acts not only on the GABA-ergic properties of ketamine but also on other inhibitory mechanisms activated by sustained stimulation of the vagus nerve. Vagal stimulation evokes inhibitory post-synaptic potential (IPSP) in inspiratory neurons in the ventral respiratory group (9). This medullary region sets the intensity of the inspiratory activity (10). NMDA receptors seem not involved in IPSPs, while GABA antagonism depresses them. Picrotoxin augments post-synaptic neuronal excitability and increases the efficacy of excitatory synaptic inputs (11), which may increase the initial amplitude of
inspiratory STD and reduce its duration. These effects suggest the role for GABA receptor mechanisms in the maintenance of STD in inspiratory activity.

In this study the post-inhibitory rebound in the respiratory rate was observed after the suppression of GABA neurotransmission by picrotoxin. This rebound is liable to originate from the structures in the rostral pons responsible for the inspiratory off-switch and results from the sensitization of pontine neurons by vagal input (3). The appearance of the post-inhibitory rebound is suggestive of the GABA receptors being involved in the regulation of integrative processes triggered by afferent inputs.

Tonic vagal stimulation in this study revealed the adaptive properties of respiratory output such as a post-stimulatory short-term depression of the inspiratory amplitude, which has not yet been systematically studied. A 20 s duration of vagal stimulation used, as opposed to 1 min in a study of Siniaia et al (3), may have caused that habituation of the response to stimulation did not always develop or the process of habituation was terminated by removal of the vagal input. In such cases, habituated inspirations during stimulation did not regain pre-stimulatory values, while after stimulation dishabituation developed. It also is possible that the intensity of stimulation used excited pulmonary high-threshold slowly-adapting receptors. Species differences should be taken into consideration as well.

We conclude that the ketamine-modulated maintenance of vagally evoked STD of inspiratory activity is antagonized by a GABA antagonist, picrotoxin. Since antagonists of the NMDA and GABA receptors alternate the level of neural excitation, the results of the present study suggest that the post-stimulatory adaptation phenomena evoked by tonic vagal stimulation depend on the state of neural excitability and are easier accomplished when the excitability is on the high side.

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REFERENCES


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