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DIVERGENT EFFECTS OF BICUCULLINE AND Picrotoxin ON KETAMINE-INDUCED APNEUSTIC BREATHING

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This study tested the potential role of inhibitory neurotransmission in the mechanism of apneustic respiration evoked by ketamine, an NMDA receptors antagonist. In the experiments performed in anesthetized, paralyzed, and ventilated cats, ketamine, in a dose of 0.5 mg/kg, was administered before and after GABA_A receptor blockade with picrotoxin or bicuculline; all agents were given intravenously. Ketamine elicited a transient, hourlong apneustic respiration consisting of an increase in inspiratory duration and a decrease in inspiratory neural amplitude. After prior administration of picrotoxin, but not bicuculline, the maximum apneustic-like prolongation of inspiration evoked by ketamine was considerably reduced. The results suggest that the GABA receptor subunits specifically sensitive to picrotoxin play a role in shaping the ketamine-induced apneustic breathing.

Key words: apneustic pattern, ketamine, picrotoxin, bicuculline

INTRODUCTION

Ketamine, a dissociative anesthetic widely used in clinical medicine, is a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptors of excitatory amino acids. Blockade of NMDA receptors by ketamine or dizocilpine leads to apneustic breathing (1). This pattern of breathing consists of a prolongation of the inspiratory phase and associated depression of the inspiratory activity while the expiratory phase remains nearly unchanged. The apneustic inspiratory phase is characterized by a long-lasting plateau of activity that is a sign of an impairment of the central inspiratory off-switch mechanism.
Several studies indicate that the apneustic-like pattern of breathing is elicited not only by NMDA antagonists but also by GABAergic and glycineergic agents. Agonists of GABA\(_{\Lambda}\) and glycine receptors strongly prolong the duration of the inspiratory phase and reduce the peak amplitude of phrenic and hypoglossal nerve activity (2, 3).

General anesthetics act via potentiation of inhibition mediated by GABA\(_{\Lambda}\) receptors. There is uncertainty of whether, beside the NMDA receptor antagonism, GABA is a target site for pharmacological effects of ketamine. Ketamine potentiates the GABA-induced chloride current (4), increases the GABA level in the brainstem, cerebral cortex (5), and cerebrospinal fluid (6), but other reports argue against GABA neurotransmission being involved in ketamine action (7, 8).

The question arises as to whether inhibition of the inspiratory off-switch mechanism and depression of inspiratory activity, described as apneustic breathing, elicited by ketamine could result from the co-involvement of GABA\(_{\Lambda}\)-ergic neurotransmission. I addressed this issue in the present study by using a competitive and non-competitive GABA\(_{\Lambda}\) receptor antagonists, bicuculline and picrotoxin, respectively, in the anesthetized cat. The results suggest that GABA\(_{\Lambda}\) receptor subtypes sensitive to picrotoxin play a role in shaping the ketamine-induced apneustic breathing.

**METHODS**

The study was performed in conformity with the EU legislation and principles in the care and use of experimental animals, and the experimental protocol was approved by a local Ethics Committee.

*Animals and instrumentation*

The experiments were performed in adult cats anaesthetized with \(\alpha\)-chloralose and urethane (40 and 1000 mg/kg, ip, respectively). Following the tracheostomy, the cats were paralysed with pipercuronium bromide (Arduan, Gedeon-Richter, Budapest, Hungary) and mechanically ventilated. Fractional end-tidal concentration of carbon dioxide and oxygen were continuously monitored with a Respiratory Gases Meter Respina IH 26 (NEC San ei Instruments, Tokyo, Japan) and kept within normal limits. A femoral vein was cannulated for injections of chemical agents and femoral artery to control the arterial blood pressure (MCK 4011S, Femed, Zabrze, Poland) and to measure the arterial blood gas content and pH (AVL Compact 2, Blood Gas assembly, Graz, Austria). Rectal temperature was maintained at 37-38° C. Both vagus nerves were cut in the mid-cervical region. The C5 phrenic nerve root was cut distally and placed on bipolar silver electrode. Phrenic nerve activity was amplified and filtered (0.5-5.0 kHz) with a NeuroLog System (Digitimer, Welwyn Garden, UK) and integrated with time constant of 70 ms. Raw and integrated phrenic nerve signals, arterial blood pressure, end-tidal CO\(_2\) and O\(_2\) were displayed on the computer screen and stored for off-line analysis with a sampling rate of 2500 Hz by an Adcjul Acquisition System (Warsaw, Poland). The variables were additionally recorded on paper with a hot-stylus Honeywell Omnilight 8 M36 Recorder (NEC San ei Instruments, Tokyo, Japan).
Neuroactive agents

Drugs and agents were administered via femoral vein. A non-competitive NMDA receptor channel antagonist - ketamine (Ketalar, Gedeon-Richter, Budapest, Hungary) 0.5 mg/kg, competitive GABA\textsubscript{A} antagonist - bicuculline methiodide 0.2 mg/kg, and non-competitive GABA\textsubscript{A} antagonist - picrotoxin (Sigma-Aldrich, Poznan, Poland) 0.2 mg/kg, were administered intravenously. All agents were dissolved in 0.9% NaCl. The doses of the agents were determined in preliminary experiments. The dose of ketamine was chosen as the lowest that evokes a clear apneustic pattern of breathing in the cat. The doses of GABA antagonists were subconvulsive and in the commonly used range.

Experimental protocol

Ketamine was injected 20 min after the completion of all experimental preparations. The respiratory pattern, turned apneustic by ketamine, reverted to normal appearance in about 1 h. At that time picrotoxin (PIC, \(n=6\)) or bicuculline (BIC, \(n=4\)) was injected. Twenty minutes after the injection of either agent, a second, same dose of ketamine was given and the respiratory effects were reinvestigated. The temporal order of the experiment took into account the long-lasting effects bicuculline (9) and picrotoxin (10). To check whether a second injection of ketamine, in itself, did not evoke a different respiratory response, in 4 experiments, the second dose of ketamine was administered 1 h 20 min after the first one without a pretreatment with GABA antagonists. Data were collected continuously during 10 min following each ketamine injection to observe the time course of apneustic breathing.

Recordings and data analysis

Respiratory variables were estimated from the phrenic neurogram. Its peak 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. Data were calculated as a percent from control before and after each pharmacological treatment and were shown as means ± SE. Differences in the longest inspiratory duration evoked by ketamine before and after picrotoxin and bicuculline were compared with Mann-Whitney's U-test. Changes in the phrenic amplitude during apneustic breathing after bicuculline and picrotoxin were compared with one-way analysis of variance followed by the Tukey post hoc test. Statistical significance was considered at P<0.05.

RESULTS

The first ketamine injection

Ketamine evoked an apneustic pattern of breathing consisting of increased inspiratory time and decreased peak of phrenic amplitude. The onset of response occurred quickly and the longest inspiration appeared within 2-10 breaths. In some animals the apneasie lasted even up to a few minutes. The mean maximum inspiratory time increased from 2.4 ±0.2 s in the control to 98.5 ±25.8 s following ketamine (mean data pooled from all 14 experiments). The time of appearance of the maximum prolongation of the inspiratory duration and the recovery varied from animal to animal. Irregular inspirations often developed. The expiratory
phase changed inappreciably. The amplitude of integrated phrenic nerve activity gradually diminished, remained depressed, and then slowly recovered. The mean minimum peak amplitude was 43.9 ±3.1% of the pre-ketamine value. The changes in respiratory pattern started to reverse after 5-10 min, returning to the baseline level in 30-60 minutes.

Effects of GABA<sub>A</sub> antagonists during eupneic breathing

Either GABA<sub>A</sub> antagonist exerted a modest but different effect on phrenic respiratory pattern (not shown). Picrotoxin stimulated, whereas bicuculline depressed, the phrenic amplitude. The inspiratory duration became longer after bicuculline, but shorter after picrotoxin. The range of these changes varied individually but overall they were statistically insignificant.

Second ketamine injection before and after pretreatment with GABA antagonists

The second dose of ketamine was administered 80 min after the first one. It was preceded by the injection of picrotoxin or bicuculline or given without any pretreatment. The results for each experimental group are presented in Table 1. Administration of picrotoxin or bicuculline did not prevent the appearance of apneustic breathing. However, in terms of the maximum prolongation of inspiration, the second dose of ketamine caused a smaller effect, be it with or without any pretreatment. This smaller effect assumed significance in the picrotoxin-pretreated animals in which the respiratory pattern did not demonstrate long inspirations seen after the first dose of ketamine. The accompanying depression of the phrenic amplitude also was significantly less after picrotoxin (Table 1). Changes in the inspiratory time and phrenic amplitude evoked by ketamine in the bicuculline-pretreated animals and by a second dose of ketamine without pretreatment were less accentuated and insignificant.

Changes in the mean arterial blood pressure

The influence of the agents used on the mean arterial blood pressure was rather meager (Table 2). Picrotoxin elevated blood pressure from 117.8 ±7.4

Table 1. Maximum inspiratory prolongation (Ti max) and minimum phrenic amplitude (A min) evoked by the first dose of ketamine (KETAMINE I) and by the second dose of ketamine (KETAMINE II) injected after bicuculline, picrotoxin or ketamine.

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<th>KETAMINE I</th>
<th>KETAMINE II</th>
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<tr>
<td>Ti max (s)</td>
<td>A min (%)</td>
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<tr>
<td>100.5 ±26.7</td>
<td>36.2 ±4.8</td>
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<td>108.1 ±78.9</td>
<td>49.0 ±7.3</td>
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<td>86.9 ±36.2</td>
<td>40.3 ±4.2</td>
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Values are means ±SE. PIC - picrotoxin (n=6), BIC - bicuculline (n=4), KET- ketamine (n=4), *P<0.05 and **P< 0.01 compared with baseline.
mmHg to 123.8 ±9.0 mmHg, while bicuculline did not change it (114.0 ±17.2 mmHg before and 113.0 ±14.3 mmHg after bicuculline). Administration of the first dose of ketamine caused small but significant (P<0.05) fall in the arterial blood pressure. The second dose of ketamine alone or with GABA antagonists pretreatment elicited insignificant blood pressure changes (Table 2).

**DISCUSSION**

In the present study, systemic administration of a small dose of ketamine (0.5 mg/kg) in the anesthetized cat produced a prolongation of neural inspiration and a depression of inspiratory amplitude, the features of apneustic respiration also known from other studies (1, 11). It is accepted that the pattern of breathing elicited by ketamine results from an impairment of transition from inspiration to expiration mediated by NMDA receptors (1). The present study showed for the first time that the respiratory effects of ketamine are modulated by GABA receptors, specifically by receptor subtypes sensitive to picrotoxin, a non-competitive GABA antagonist. Both depression of the inspiratory activity and apneustic prolongation of the inspiratory phase were antagonized by picrotoxin.

Ketamine action through the GABA system remains controversial. The lack of GABA receptor agonistic properties of ketamine, suggested in some reports (7, 8, 12), was based on ineffectiveness of bicuculline to reverse ketamine-evoked effects. However, picrotoxin has not been tried to block the action of ketamine in any of the above cited studies. On the other hand, at least a partial transmission of ketamine action by GABA receptors has been suggested by others (13). This is in line with the finding that ketamine enhances GABA receptor-mediated current *in vitro* (4) and *in vivo* (14). In a study on hippocampal slices, ketamine has reduced the excitation mediated by NMDA receptors and also enhanced the inhibition mediated by GABA receptors (15). If the same was true for the synaptic transmission in respiratory neurons, the increase in ketamine-induced GABA-mediated inhibition might be mitigated by picrotoxin.

The present study revealed that GABA antagonists changed the respiratory pattern elicited by ketamine in a different way. The reason of dissimilar respiratory effects of picrotoxin and bicuculline results probably from different

<table>
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<th>Control</th>
<th>Effect</th>
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<tr>
<td>KET I</td>
<td>113.4 ±3.5</td>
<td>108.9 ±3.8*</td>
</tr>
<tr>
<td>KET II</td>
<td>112.0 ±4.1</td>
<td>109.4 ±5.6</td>
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<tr>
<td>KET II after PIC</td>
<td>123.8 ±9.0</td>
<td>121.4 ±12.8</td>
</tr>
<tr>
<td>KET II after BIC</td>
<td>113.0 ±14.3</td>
<td>103.9 ±12.8</td>
</tr>
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Values are means ±SE. KET I - first dose of ketamine, KET II - second dose of ketamine, PIC - picrotoxin, BIC - bicuculline *P<0.05 compared with control.
pharmacological properties of bicuculline, a competitive GABA\(\text{A}\) receptor antagonist, and picrotoxin, a non-competitive GABA receptor antagonist (16). The effect of a given GABAergic substance depends on its binding to specific GABA\(\text{A}\) receptor subunits, which are distinct for picrotoxin and bicuculline (17). Additionally, picrotoxin is an antagonist of GABA\(\text{C}\) receptors and inhibits GABA-mediated activation of the receptor \(\rho\) subunit by a competitive mechanism (18). This type of relatively recently described GABA receptors has been discovered first in the retina and soon afterward in the central nervous system (18). GABA\(\text{C}\) receptors are picrotoxin sensitive and bicuculline insensitive. Although there is no information yet, on whether or not ketamine engages the \(\rho\) subunit of GABA receptors, the lack of consistent effects of bicuculline and significant effects of picrotoxin on apneustic breathing points to the possible inhibition of GABA\(\text{C}\) receptors by ketamine.

During eupneic breathing, the GABA antagonists bicuculline and picrotoxin exhibit different neurophysiological effects on respiratory neurons (19). Picrotoxin produces a dose-dependent increase in the rate of discharges and induces tonic activity during the silent phase in inspiratory and expiratory neurons with only a small increment of inspiratory activity (11, 20, 21). In contrast, bicuculline does not influence tonic activity, acts on the active phase of inspiratory and expiratory neurons and accelerates their discharge rate (19). An augmentation of tonic activity by picrotoxin probably facilitates the tonic excitatory drive to respiratory neurons in eupnea. Tonic excitatory input during the inspiratory phase (11, 20, 21) is mediated by NMDA receptors. Exogenous stimulation of glutaminergic neurotransmission by NMDA increases the firing rate with appearance of a tonic type of discharges in the silent period of respiratory neurons activity (10). This tonic activity is reduced by a blockade of the NMDA mechanism with the competitive NMDA receptor antagonist AP-5 (10). Assuming that ketamine antagonizes the transmission of tonic drives by NMDA receptors, as does the AP-5, one may speculate that such drives suppressed by ketamine are relieved or restored by picrotoxin. This, in turn, might be responsible for stimulation of the inspiratory off-switch and an augmentation of inspiratory amplitude.

Ketamine interferes with various neurotransmitter systems. The dose of ketamine used in the present study is used clinically to produce analgesia (22). Analgetic properties of small doses of ketamine depend on the central cholinergic system and acetylcholine (ACh). Ketamine binds selectively to nicotinic ACh receptors (12), as it does to NMDA receptors, and inhibits muscarinic receptors (23). A decrease in ACh release is a likely cause of respiratory rate slowing (24). Picrotoxin, in itself, increases the spontaneous release of ACh and that inhibited by GABA (25), by blocking GABA\(\text{A}\) and possibly GABA\(\text{C}\) receptors (26). Thus, picrotoxin might reverse the ketamine-induced apneustic breathing through an interference in GABA - central cholinergic mechanisms.
A second dose of ketamine in the present study had a smaller apneustic effect, which indicates a possible desensitization of the inspiratory off-switch mechanism to ketamine. The mechanism of such desensitization is unknown. It might be linked to changes in the brain content of ketamine accompanying a rapidly developing tolerance (27). It also is possible that the blockade of NMDA receptors on respiratory neurons by the first dose of ketamine persisted longer than the respiratory symptoms and the receptors were less apt to respond to the antagonist again.

In conclusion, the study showed that the apneustic pattern of respiration evoked by ketamine contained a GABAergic component mediated by the GABA receptor subunits sensitive to picrotoxin.

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REFERENCES


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