Augmentation of Hypoxic Respiration after Brief Hyperoxia in the Anesthetized Cat: Putative Function of GABA\(_{A}\) Neurotransmission

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Bicuculline • Cats, respiration in • GABA antagonism • GABA neurotransmission • Hypoxic respiratory response • Oxygen • Phrenic nerve activity

Abstract
In this study, we attempted to determine the role of GABA neurotransmission in augmentation of hypoxic respiration by antecedent hyperoxic breathing. The experiments were performed in anesthetized, paralyzed and vagotomized cats divided into control and bicuculline (a GABA\(_{A}\) receptor blocker)-injected groups. The experimental protocol consisted of exposing the animals to successive hypoxic-hyperoxic-hypoxic conditions. Respiration was assessed using phrenic electroneuromograms, from which the peak phrenic height, a surrogate of the tidal volume component, and respiratory rate were obtained, and their product, the respiratory minute output, was calculated. We found that prior hyperoxic ventilation increased the subsequent respiratory response to hypoxia by an average of 23.5%, compared with the preoxygen response. This increase was driven by volume respiration. The biphasic character of the hypoxic respiratory response, consisting of stimulatory and depressant phases, was sustained. Bicuculline abolished the augmentative effect on hypoxic respiration of prior hyperoxia, which suggests that oxygenation induces GABA\(_{A}\)-mediated hyperexcitability of respiratory neurons, possibly by the liberation of reactive oxygen species. We concluded that GABA neurotransmission is pertinent to the effect of hyperoxia on hypoxic respiratory reactivity.

Introduction

It was previously established that a short period of hyperoxic breathing increases the subsequent ventilatory response to hypoxia in conscious spontaneously breathing humans [13] and rats [11]. There is no agreement concerning the mechanisms underlying this phenomenon. While it has been postulated that elaboration of central neurotransmitter pathways by \(O_2\) is involved in the control of the respiratory neuronal network, ventilatory hypoxic augmentation has variably been ascribed to activation of the brain glutamatergic [13] or nitrergic mechanism [11].

Apart from neurotransmitter mechanisms, the stimulatory effect on ventilation of \(O_2\) may have a consciousness-related component. The state of wakefulness affects
hyperoxic augmentation of the hypoxic ventilatory response in opposing manners. On the one hand, noxious stimuli, such as hypoxia and hypercapnia, the latter being added to hypoxic gas inspiratory mixtures to prevent the arterial CO2 partial pressure from falling during hypoxic hyperventilation [11, 13], are sensed by higher brain structures which facilitate hyperventilation. On the other hand, hypoxia reduces basal bioelectric cortical activity, particularly in the alpha frequency range [31], and the effect may radiate to the brain stem respiratory neuronal network [7] and reduce the latter’s responsiveness. The net ventilatory response is the balanced product of these two opposing dynamics.

The central stimulating component of hyperoxia has been described in conscious humans [1] and animals [9]. In a state of wakefulness, the component is powerful enough to override the inhibitory effect on ventilation of O2 mediated by carotid body chemoreceptors, but is sensitive to anesthesia, under which the latter becomes unmasked [9]. In contrast to the consciousness-related cortical component, central neurotransmitter influences on respiration should not be abolished by anesthesia. Therefore, anesthesia may help single out the neurotransmitter aspect of the stimulatory effect on the hypoxic respiratory response of O2. In the present study, we addressed this issue by comparing the hypoxic respiratory response before and after a short-term period of ventilation with O2 in anesthetized cats. Additionally, we examined the effect of bicuculline, a GABA receptor antagonist, on the postoxygen hypoxic respiratory response. The rationale for using GABA receptor antagonism was that glutamine, whose concentration has been reported to increase in the blood due to hyperoxia [13], is the precursor of both glutamate and GABA. GABA-mediated neurotransmission may assume an excitatory character under certain cellular conditions, such as depolarizing shifts in the transmembrane Cl- gradient [35]. Cl- gradient shifts are promoted by reactive oxygen species, formed as a result of changes in the partial pressure of O2 used in this study. We found that a period of ventilation with O2, which preceded the hypoxic bout, markedly augmented subsequent hypoxic respiration, and that this augmentation was abolished by use of a GABA antagonist.

Materials and Methods

Animals and Surgical Procedures

The experiments were performed on 24 adult cats of either sex anesthetized with 35 mg/kg i.p. chloralose and 800 mg/kg urethane. This combination of anesthetics is commonly used in animal studies due to its minimal influence on respiration and respiratory chemoreflexes. Additionally, urethane, a major component of the combination, only modestly affects multiple neurotransmitter systems, rather than any specific one [12], thus lessening the likelihood of changes in the balance of the various neural pathways. The animals were divided into a control group (13 cats with a mean weight of 3.18 ± 0.12 kg) and a bicuculline group (11 cats with a mean weight of 3.37 ± 0.19 kg). Animals of both groups were placed in a supine position, tracheostomized, paralyzed with 0.1 mg/kg/h pancuronium bromide (Pavulon) and artificially ventilated on room air. Tidal volume and frequency parameters of the respirator were adjusted to maintain the arterial oxygen (PaO2) and arterial carbon dioxide (PaCO2) partial pressures within normal ranges. Both vagus nerves in the neck were isolated and transected. The C5 root of the phrenic nerve was exposed, transsected and desheathed, and placed on bipolar silver recording electrodes. The preparation included cannulation of both the femoral artery and vein. The rectal temperature was maintained at approximately 37.5°C with a heating pad. All cats were maintained in accordance with accepted standard principles in the care and use of animals. The institutional Ethics Committee approved the study (permit No. 75/2000).

Experimental Protocol

The experimental protocol was the same for both the control and bicuculline groups and consisted of a series of successive exposures to various gas conditions, i.e. 3 min of hypoxia (7% O2 in N2), 10 min of hyperoxia (100% O2) and repeated exposure to hypoxia. The recovery interval after the first hypoxic test was about 3 min, and the switch from hyperoxia to the second period of hypoxia was made within 30 s. Bicuculline (bicuculline methiodide, Sigma-Aldrich, Steinheim, Germany), a GABA receptor antagonist, was dissolved in 0.9% NaCl and injected intravenously as a bolus of 0.1 mg/ml/kg. A dose of bicuculline was chosen which produced the desired antagonism of GABA-mediated respiratory effects but was below the convulsive threshold. The dose was based on available data from the literature. In the cat, the maximum effect of bicuculline on the phrenic output has been found to be, on average, at a dose of 0.091 mg/kg, and on the convulsive threshold at 0.127 mg/kg [24], while 0.1 mg/kg intravenous bicuculline suffices to reverse GABA-induced respiratory depression [20]. Bicuculline was injected once at the beginning of each experiment in the bicuculline group after the control recordings had been taken, and the protocol was begun 15 min later. The temporal order of the bicuculline experiment was thus arranged because it takes several minutes for bicuculline to develop its full effect on the central nervous system after intravenous administration [24]; thereafter, the effect is sustained for up to several hours [2]. All animals of the bicuculline group underwent the exact same number of experimental runs and order of the experimental conditions to avoid confounding effects on respiration that could arise from a different number of hypoxic tests or prolonged experimental time. Control injections of 0.9% NaCl alone produced no effect on the respiratory variables recorded.

We used a steady-state type of hypoxia with a stepwise switch to the hypoxic gas mixture. Gas mixtures were given in a 20-liter bag connected to the inspiratory port of the respirator. Any possible fluctuations in the end-tidal CO2 concentration after connecting the bags with the gas mixtures were minimized by slight adjustments of the respirator’s parameters, so that the preset normocapnic level was closely maintained throughout the experimental period. The end point of hypoxia was set as the time when hypoxic respiratory depres-