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Augmentation of Hypoxic Respiration after Brief Hyperoxia in the Anesthetized Cat: Putative Function of $GABA_A$ Neurotransmission

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Key Words

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 electroneurograms, 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

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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 CO_2 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 O_2 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 O_2. In the present study, we addressed this issue by comparing the hypoxic respiratory response before and after a short-term period of ventilation with O_2 in anesthetized cats. Additionally, we examined the effect of bicuculline, a $GABA_A$ receptor antagonist, on the postoxygen hypoxic respiratory response. The rationale for using $GABA_A$ 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. $GABA_A$-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 O_2 used in this study. We found that a period of ventilation with O_2, 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 α-chloralose and 800 mg/kg urethane (i.p.). 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 (PaO_2) and arterial carbon dioxide ($PaCO_2$) partial pressures within normal ranges. Both vagus nerves in the neck were isolated and transected. The C_5 root of the phrenic nerve was exposed, transected 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% O_2 in N_2), 10 min of hyperoxia (100% O_2) 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 $GABA_A$ 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 $GABA_A$-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 CO_2 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-

sion after the initial stimulation caused the phrenic output to decline back to the baseline level, which usually took 2.5–3.0 min. By choosing such an end point, the hypoxic runs were of relatively short duration, which was desirable because of the need to repeat the hypoxic runs. Longer durations of hypoxia and ensuing profound respiratory depression can destabilize an animal's condition, make the recovery prolonged or unattainable in full, and can thus confound comparisons among sequential hypoxic runs.

Using this study paradigm, changes in the gas condition were completed within about 30–40 min. It was assumed that no major fluctuations in the level of the long-acting anesthesia would occur which could have appreciably influenced respiration during such a short time. Stability of the anesthesia was additionally checked by observing pupil size. Since no signs of inadequate anesthesia were detected, no supplemental anesthesia was given during the experimental period.

Measurements and Data Analysis

Respiratory output profiles were recorded in the form of phrenic nerve electroneurograms. The raw phrenic nerve activity was amplified, filtered and integrated with a time constant of 100 ms to obtain its moving time averages (Digitimer-Neurolog System, Welwyn Garden City, UK). End-tidal O_2 and CO_2 concentrations in the expired gas were sampled in the trachea (Respina IH26, NEC San-ei Instruments, Tokyo, Japan), and arterial blood pressure was continuously monitored with a strain gauge transducer and an electromanometer (MCK4011S, Femed, Zabrze, Poland). All recordings were displayed on a strip-chart hot-stylus polygraph (Honeywell-Omnilight 8M36, NEC San-ei Instruments). Arterial blood was drawn for measurement of blood gas content and pH before and after changes in the inspired gas mixture (Compact 2 blood gas analyzer, AVL List, Graz, Austria).

The peak amplitude, taken as a surrogate of the phrenic motor output descending to the diaphragm and therefore of tidal volume [16], and the duration of the total respiratory cycle (T_{TOT}) from the onset of activity to its next onset were measured and quantified from the integrated phrenic activity trace. The respiratory frequency was derived as $60/T_{TOT}$ from the latter variable. Minute phrenic output, an index of minute ventilation, was calculated as the product of phrenic amplitude and frequency.

The mean value of each variable was computed for a period of 3 full respiratory cycles every 30 s during hypoxic exposure and once each at baseline, at the end of hyperoxic exposure and after the bicuculline injection. Data are expressed as the percentage change from the preceding baseline level, and the group mean ± SE was calculated. Differences between the pre- and postoxygen hypoxic responses were assessed using two-way analysis of variance (ANOVA) for repeated measures. In the analysis, the pre- and postoxygen conditions were between-group factors, and the progressing 30-second time points of hypoxic exposure were a repeated-measure within-group factor. Peak hypoxic respiratory increases before and after ventilation with O_2, the effects of O_2 and bicuculline alone, and the arterial blood pressure between the peak and nadir of the respiratory hypoxic response were compared using a paired t test. A p value of <0.05 was considered significant for all statistical comparisons.

Results

Preoxygen Hypoxic Respiratory Response in the Control Group

The minute phrenic output showed a typical biphasic response to hypoxia (fig. 1A). The increase in this output peaked 1.0 min after the onset of hypoxic exposure and reached 142.9 ± 12.4% of the baseline level. Stimulation of the phrenic output was followed by a gradual decline that approached the baseline level 2.5 min from the start of hypoxia. The increase in the minute phrenic output was entirely driven by volume respiration, as expressed by the phrenic amplitude (fig. 1B). Changes in the phrenic output and amplitude over the time course of the response were significant ($p < 0.03$).

Postoxygen Hypoxic Respiratory Response in the Control Group

Ventilation with O_2 for 10 min decreased the minute phrenic output to 86.4 ± 4.2% and phrenic height to 85.9 ± 3.3% of the respective preoxygen levels ($p < 0.05$), with no change in frequency (100.1 ± 1.7%). The hypoxic test repeated after the period of hyperoxia showed an augmented respiratory response that otherwise was qualitatively similar to that measured before oxygen administration. The minute phrenic output and phrenic amplitude were sustained at higher levels than before hyperoxia with small adjustments from the aspect of respiratory frequency (fig. 1). The peak increase in the minute phrenic output amounted to 166.4 ± 14.9%, which was a significant ($p < 0.05$) increase over that noted in the preoxygen hypoxic test, as outlined above. The increased phrenic output still amounted to 145.1 ± 20.7% of the baseline level at 2.5 min, the end point of the test, which resulted in a slower hypoxic falloff after oxygen administration (fig. 1A). A significant interaction was observed between the time of hypoxic exposure and the oxygen condition group (fig. 1A, B) ($p < 0.001$).

Effects of Prior O_2 on the Hypoxic Respiratory Response in the Bicuculline Group

A pharmacological manipulation was performed to gain insight into the mechanism by which O_2 augments the subsequent hypoxic respiratory response. Injection with the specific $GABA_A$ receptor antagonist, bicuculline, was followed by a gradual modest increase in minute phrenic output that peaked at 3–4 min, amounting to 118.9 ± 9.3% of the preinjection value ($p < 0.03$). A modest increase in respiration after bicuculline alone conforms to results of a previous study that employed intra-

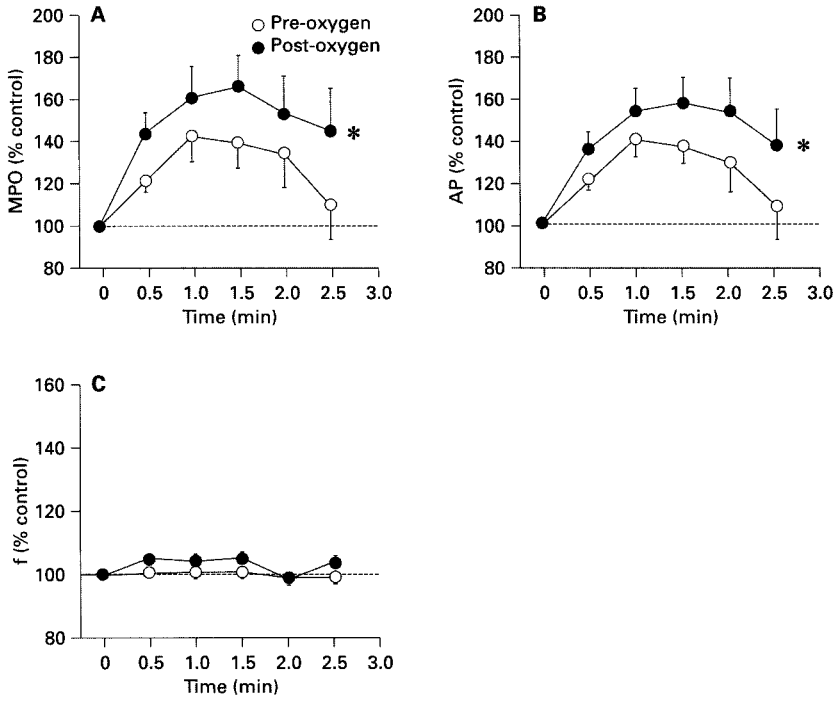

Fig. 1. Time course of the hypoxic respiratory response in control cats before (open circles) and after (target circles) ventilation with oxygen. **A** Minute phrenic output (MPO). **B** Peak phrenic amplitude (AP). **C** Breathing frequency (f). Data are given as the mean ± SE, expressed as a percentage of the baseline, and were analyzed with two-way ANOVA for repeated measures. Statistical interaction between time and groups is shown. * $p < 0.001$.

venous injections of this agent at a similar dose [30]. The increase, which was due entirely to the higher phrenic amplitude, receded thereafter to a mere 104.4 ± 7.6% of the baseline 15 min after the bicuculline injection, at which time the first hypoxic test began. The hypoxic respiratory response after bicuculline was closely reminiscent of that in the control group, with its biphasic character, stimulation dependent on the phrenic amplitude and the peak minute phrenic output increase of 139.2 ± 9.6% at 1.5 min.

Ventilation with O_2 for 10 min with a background of bicuculline caused an insignificant increase in the minute phrenic output that amounted to 106.1 ± 7.4% of the baseline. In contrast to the control group, there was virtually no augmentation of the minute phrenic output or any of its components during the hypoxic test repeated after oxygenation (fig. 2). The course of the postoxygen hypoxic respiratory response nearly overlapped with that of the preoxygen one, showing a peak minute phrenic output of 144.7 ± 9.3% at 1.5 min and a later falloff toward the baseline level. While there was a significant effect of time of hypoxic exposure on the minute phrenic output and its amplitude ($p < 0.003$), no interaction between time and group was observed.

Arterial Blood Gas Tensions, pH and Blood Pressure

Changes in arterial blood gas tensions and pH across the experimental conditions studied, other than those in the PaO_2 resulting from the implementation of the hypoxic-hyperoxic gas mixtures, were insignificant (table 1). In the control group, the mean baseline PaO_2 was 86.6 ± 3.2 mm Hg, and it fell to 20.7 ± 1.0 mm Hg during hypoxia before the application of O_2, and then to 22.6 ± 1.3 mm Hg in the postoxygen hypoxia. Oxygenation increased the PaO_2 to an average of 378.7 ± 16.1 mm Hg. There were no major changes in $PaCO_2$ or arterial pH, which remained in the normal range. Bicuculline alone did not appreciably change the blood gas or pH indices. The profile of their changes resulting from the gas mixtures employed after bicuculline remained closely similar to that in the control group.

Arterial blood pressure changes in the control and bicuculline groups are presented in table 2. On the whole, these changes were not remarkable and were basically similar in both groups. Systolic pressure tended to increase at the peak of respiratory stimulation during preoxygen hypoxia, with no major change in diastolic pressure. During the nadir of the respiratory falloff at the end of hypoxic exposure, both systolic and diastolic pressures

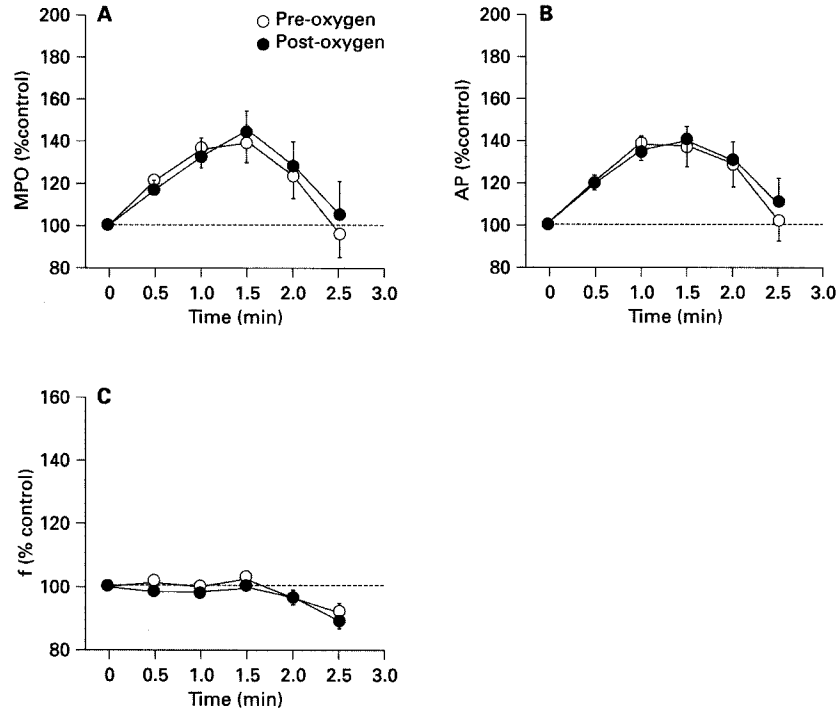

Fig. 2. Time course of the hypoxic respiratory response in bicuculline-injected cats before (open circles) and after (target circles) ventilation with oxygen. **A** Minute phrenic output (MPO). **B** Peak phrenic amplitude (AP). **C** Breathing frequency (f). Data are given as the mean ± SE, expressed as a percentage of the baseline. Bicuculline abolished the augmentative effect of prior O_2 on the subsequent hypoxic respiratory response, as seen in figure 1.

Table 1. Arterial blood gas tensions and pH across the experimental conditions studied in the control and bicuculline groups

	Control group				Bicuculline group			
	baseline	hypoxia I	hyperoxia	hypoxia II	baseline	hypoxia I	hyperoxia	hypoxia II
PaO_2, mm Hg	86.6±3.2	20.7±1.0	378.7±16.1	22.6±1.3	81.5±2.9	21.0±1.0	353.3±20.9	21.8±0.9
$PaCO_2$, mm Hg	36.5±1.9	35.3±1.7	37.2±1.5	34.7±2.5	38.8±1.7	37.0±2.6	39.9±2.1	37.4±3.0
pHa	7.32±0.01	7.33±0.01	7.31±0.02	7.34±0.02	7.30±0.02	7.33±0.01	7.29±0.02	7.31±0.02

Values are means ± SE. pHa = Arterial pH.

Table 2. Arterial blood pressure changes in the control and bicuculline groups

	Control group						Bicuculline group					
	baseline	hypoxia I		hyperoxia	hypoxia II		baseline	hypoxia I		hyperoxia	hypoxia II	
		peak	nadir		peak	nadir		peak	nadir		peak	nadir
SP, mm Hg	135.0±7.6	143.5±9.2	109.6±8.5*	134.0±6.4	133.3±5.3	106.0±8.3*	138.7±6.5	142.9±5.9	105.2±9.4*	137.8±4.9	132±5.3	101.1±9.5*
DP, mm Hg	80.0±4.8	78.6±5.9	60.8±6.3*	83.4±5.7	76.6±5.4	55.4±6.0*	88.6±6.0	84.4±4.3	59.3±5.0*	87.7±4.9	78.5±3.0	53.5±6.4*

Values are means ± SE. SP = Systolic pressure; DP = diastolic pressure. * $p < 0.05$ compared to the corresponding peak level.

significantly decreased compared with the peak hypoxic level ($p < 0.05$). The decrease was below the baseline pre-hypoxic level, but blood pressure still remained within reasonable limits. Blood pressure recovered to the baseline level during oxygenation. During postoxygen hypoxic exposure, the profile of blood pressure changes was similar to that in the preoxygen setting.

Discussion

This study demonstrates that a period of ventilation with O_2 strengthens the respiratory response to subsequent hypoxia. The effect was mostly mediated by enhanced neural motor output to the diaphragm (i.e. volume respiration, the surrogate of which was the peak height of phrenic activity) and was inhibited by use of a $GABA_A$ antagonist. The enhancement of hypoxic respiration by prior O_2 corroborates the findings of previous studies in conscious humans [13] and rats [11]. The increment in the minute respiratory output in our study amounted to an average of 23.5%, lower than the 31% in humans, which was also driven by higher-volume respiration, and the 47% in rats noted in the aforementioned studies. Nevertheless, the similarity between these and previous findings is remarkable in view of the different experimental designs. We elected to separate two identical hypoxic runs by an interval of ventilation with O_2, so that the second hypoxic test could be referenced to the first control test in the same animal, rather than to a random assignment of breathing oxygen or room air. The latter design, particularly with the small populations used in previous studies [11, 13], possibly produced the fortuitously higher average counts of respiration in the oxygen group and thus does not necessarily truly reflect the augmenting effect of O_2 on the hypoxic response in an individual. Each animal being its own control averted the possibility of such a false-positive result. The present study was performed on cats in a state of anesthesia which were paralyzed and ventilated, and with no addition of CO_2 to the airways. Such a design allowed a few plausible mechanisms that might play a part in O_2-related respiratory augmentation to be discounted.

Higher brain structures are operative in shaping the response to hypoxia, a stressful stimulus, in a state of wakefulness. This is evident in awake humans [13] and also in conscious rats, in which the depressant phase of the response, characteristic of hypoxia, is nullified shortly after its initiation by an overriding secondary increase in ventilation [11]. Enrichment of hyperoxic mixtures with CO_2, used to maintain a constant level of $PaCO_2$, must be more intense during postoxygen hypoxic ventilation augmentation, and may produce a noxious perception which would promote hyperventilation. Anesthesia interrupts cortical influences descending to the brain stem respiratory neuronal network or directly to respiratory motoneurons [7]. Therefore, consciousness-related effects or possible changes in cortical bioelectrical activity induced by chemical stimuli [31] could not realistically underlie the augmentation of hypoxic respiration by O_2 observed in the present study.

This augmentation cannot be ascribed to the vagally mediated neural respiratory traffic in the open-loop condition in which the vagus nerves are severed. Vagal C-fiber afferents might play a part in the phenomenon, since they are engaged in hypoxia-induced increases in the end-expiratory lung volume and consequently in end-expiratory diaphragmatic activity [38]. Such increases, which might lower the tidal volume response to hypoxia, are countered by hyperoxia [8]. CO_2 itself may increase volume respiration by way of central chemoreceptors and also by a vagally mediated inhibitory effect on pulmonary stretch receptors [4, 5].

The ability of oxygen to influence hypoxic respiration means that the effect of O_2 is sustained after its withdrawal. Neither the exact time length nor the determinants of the action of O_2 are clear. There are reports suggesting that relief from the hypoxemic dysfunction of the central nervous system persists for at least 30 min after O_2 discontinuation [23], although others have failed to note extension of the effect of O_2 beyond the period of its administration [10]. On the other hand, others have reported that hyperoxic hyperventilation was sustained for at least 15 min after O_2 withdrawal [1]. The sustained effect suggests that O_2 can modify the central neurotransmitter pathways intimately engaged in respiratory regulation.

One such pathway is GABA neurotransmission. GABA is derived from the amino acid glutamine, from which glutamate is also formed. It is known that both GABA and glutamate are active regulators of the central respiratory drive in inhibitory and excitatory manners, respectively, and that both are elaborated during changes in the PaO_2 [18]. Reductions in the PaO_2 cause the rapid release of GABA into the extracellular space in a variety of neuronal preparations [3, 28, 35]. The increased GABA content affects hypoxic ventilatory depression [15]. GABA neurotransmission mitigates the excitatory action of glutamate, which is centrally released as a result of carotid body chemoreceptor excitation by hypoxia [14].

Prior O_2 breathing increases the glutamine concentration in peripheral blood [13]. It is possible that the brain glutamate content increases as well, which in turn can lead to an excitatory effect on respiration.

It seems, however, that increased formation of excitatory glutamate does not have to be invoked to explain the augmenting effect of O_2 on hypoxic respiration. The explanation may lie in disruption of brain GABA metabolism by oxidative insults. Such insults are caused by reactive oxygen species which accumulate during hyperoxia, but are also liberated by hypoxia [39]. Reactive oxygen species increase the release and decrease the uptake of GABA, but lead to compromising changes in the $GABA_A$ receptor-gated Cl^- channel function that underlies neuronal inhibition [33]. Reactive oxygen species increase intracellular Cl^-, which decreases the transmembrane Cl^- gradient, normally negative relative to the outside of the cell [34]. In pyramidal neurons, the increase in intracellular Cl^-, accompanied by decreased $GABA_A$ responses, is especially evident in an ischemia-reperfusion setting [35], which bears a functional resemblance to the hypoxia-hyperoxia scheme used in the present study. The Cl^- gradient drives Cl^- influx by GABA and thus is essential for the expression of GABA-mediated inhibitory responses. Reversal of the Cl^- gradient results in depolarizing responses to GABA. Such responses, which may become excitatory, have been reported as early as a few minutes after neuronal trauma in vitro as a result of a positive shift in the Cl^- gradient [32] and are particularly observable in neurons that are repeatedly stimulated or exposed to GABA [36], features plausibly present in the current study. The in vivo presence of depolarizing shifts in the Cl^- equilibrium potential and reductions in GABA-mediated inhibition were confirmed in cerebral ischemia in rats [25]. The increase in intracellular Cl^- was prevented by picrotoxin, another $GABA_A$ receptor antagonist [17]. In addition to alterations in the Cl^- gradient, the ischemia-reperfusion setting activates the arachidonic acid cascade, which compromises $GABA_A$ receptor function and leads to neuronal hyperexcitability, alongside enhanced glutamatergic activity [35]. If the hypoxia-hyperoxia-hypoxia cycle of the present study design can derange the function of GABA-loaded interneurons engaged in generating respiratory motor activity toward the hyperexcitatory state, which would lead to an augmentative effect of hyperoxia on hypoxic respiration, then bicuculline would abolish the effect by preventing such changes.

In the present study, bicuculline administration was not associated with a reduction in hypoxia-induced respiratory depression, which may seem at odds with the plausible role of GABA in central respiratory responses to chemical stimuli [18]. Elaboration of GABA has been established in the mechanisms of central hypoxic depression in newborn animals [15] and in experimentally evoked conditions, such as brain hypoxia resulting from carbon monoxide inhalation [24], hypothermic piglets [40] and obese rats [21]. Under those conditions, bicuculline administration reverses the late hypoxic respiratory decline by a substantial amount. However, the role of GABA is questionable in normal humans, as the neurotransmitter did not seem to affect the chemical drive [6], and also in normal rats, as bicuculline failed to affect the respiratory responses to hypoxia or hypercapnia [21]. The lack of modulation of respiratory hypoxic falloff by $GABA_A$ antagonism we observed is in accordance with the latter concept.

The findings of this study are not readily reconcilable with previous work on the stimulatory effect of hyperoxia on hypoxic respiration. In a human study [13], the content of GABA measured in the blood increased, alongside glutamine, in 5 of 10 subjects. The increase did not assume statistical significance, which was likely due to the small sample size. Because $GABA_A$ antagonists cannot be used in human subjects, the issue remains unresolved, but the role of reversed GABA neurotransmission, as outlined above, could possibly be a viable option. In a rat study [11], nitric oxide (NO) was implicated in the mechanisms of hypoxic stimulation after hyperoxia. However, although O_2 is a cofactor for NO synthesis, the increase in neuronal NO synthase or NO concentration under normobaric conditions, as opposed to hyperbaric conditions, was not significant [37]. Central NO caused excitatory modulation of the hypoxic ventilatory response [26] in some studies, but inhibited neurons in the areas involved in respiratory control in others [22]. Moreover, the inhibitory effect of NO is mediated by $GABA_A$ neurotransmission, which is incompatible with abolishment of the stimulatory effect of hyperoxia on hypoxic respiration by bicuculline found in the present study. NO facilitates release of other neurotransmitters, such as catecholamines, acetylcholine and neuroactive amino acids, all of which are engaged in chemical respiratory control [19]; this may confound the assessment of its effects. Clearly, the role of NO in neuronal respiratory networks requires additional studies.

There is, however, a dedicated link between NO and bicuculline-induced effects on cerebral blood flow through which bicuculline may influence respiration, independently of the $GABA_A$ receptor-gated Cl^- channel. Bicuculline, at convulsive doses of 0.4–0.9 mg/kg intra-

venously, causes an abrupt several-fold increase in cerebral blood flow in the rat, accompanied by markedly increased tissue oxygenation, due to vasodilatation that follows brain tissue hypermetabolism and CO_2 retention [29]. The hyperemia caused by bicuculline is mediated by NO, as it is blocked by an inhibitor of NO synthase; the blockage was reversed by an excess of *L*-arginine substrate [27]. The vascular effect of bicuculline counters hyperoxic cerebral vasoconstriction and may mitigate the hyperventilation caused by hypoxia. In the present study, in which subconvulsive doses of bicuculline were used, neither the cortical bioactivity nor cerebral blood flow was monitored. Nevertheless, any coexisting effect of bicuculline on the cerebral vasculature may have played a part in abolition of postoxygen hypoxic respiratory augmentation. The precise determinants of this augmentation could not be discerned in this study and thus require alternative study designs.

We conclude that a period of hyperoxic breathing augments subsequent hypoxic respiratory reactivity in an anesthetized state in a manner comparable with that in the conscious state. Abolition of respiratory augmentation by use of a GABA antagonist suggests that GABA-mediated hyperexcitability of respiratory neurons might emerge, possibly induced by depolarizing shifts in the Cl^- gradient caused by reactive oxygen species, after oxygenation. Our findings indicate the sustained influence that O_2 has on hypoxic respiration and add to the previously reported glutamatergic and nitrergic pathways involved in this influence, which suggests that multiple mechanisms are involved in the effect of O_2. Irrespective of the exact determinants of this effect, the possibility arises that brief episodes of hyperoxic breathing, rather than prolonged O_2 supplementation, might enhance hypoxic respiration, and thus the delivery of oxygen to tissues in respiratory pathologies underlain by hypoxia. The potential therapeutic implications of brief hyperoxic breathing for hypoxic respiration remain to be explored.

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