

The Role of Gamma-Aminobutyric Acid and Glutamate for Hypoxic Ventilatory Response in Anesthetized Rabbits

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YELMEN, N.K. *The Role of Gamma-Aminobutyric Acid and Glutamate for Hypoxic Ventilatory Response in Anesthetized Rabbits.* Tohoku J. Exp. Med., 2004, **203** (3), 219-232 — Acute hypoxia produces an increase in ventilation. When the hypoxia is sustained, the initial increase in ventilation is followed by a decrease in ventilation. Hypoxia causes changes in brain neurotransmitters depending on its severity and durations. The purpose of this study was to investigate the role of gamma-aminobutyric acid (GABA) and glutamate for hypoxic ventilatory response in rabbits. The experiments were performed in peripheral chemoreceptors intact and denervated rabbits anesthetized with Na-pentobarbitate. For intracerebroventricular (ICV) injections of reagents in each animal, cannula was placed in left lateral cerebral ventricle by stereotaxic method. After ICV injection of GABA (0.48 mg/kg), air breathing in both groups caused a depression of respiratory activity. On the other hand, after ICV injection of GABA, breathing of hypoxic gas mixture (8% O₂-92% N₂) in both groups produced the hypoxic hyperventilation. After ICV injection of GABA, blockade of GABA_A receptors with bicuculline (0.2 mg/kg) did not prevent the hypoxic hyperventilation. In contrast, after ICV GABA injection, blockade of glutamate NMDA receptors with MK-801 (0.09 mg/kg) completely abolished the hypoxic hyperventilation observed while the animals were breathing hypoxic gas mixture. Our findings suggest that ICV injection of GABA causes respiratory depression in normoxic conditions, and that it increases ventilation in hypoxic conditions with or without peripheral chemoreceptor impulses by increasing glutamate. ——— GABA; hypoxia; control of breathing; bicuculline; MK-801
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Gamma-aminobutyric acid (GABA), the most important inhibitory neurotransmitter in the central nerve system, is produced from glutamate by the effect of the enzyme, glutamate decarboxylase (Wu 1976). GABA and glutamate have adverse effects on the neuronal activity (Mueller et al. 1982). It has been shown that GABA receptors are localized in the brain stem and cardiorespiratory nuclei (Taveira-Da Silva et al. 1987).

Centrally administered GABA and GABAergic agonists depress respiration in normoxic conditions (Yamada et al. 1981, 1982; Kneussl et al. 1986; Taveira-Da Silva et al. 1987). Respiratory depression may be caused by disappearance of the activity of dorsal and ventral respiratory group of neurons as a result of neuronal hyperpolarization (Anderson et al. 1980; Champagnat et al. 1982; Bennet et al. 1987). Central administration of bicuculline, a GABA_A receptor antagonist, causes an increase in ventilation (Melton et al. 1990; Soto-Arape et al. 1995). These results suggest that GABA is released from synapses of the neuronal circuits related to central respiratory activity in the central nervous system (CNS). Therefore, GABAergic pathways are important in the regulation of spontaneous respiratory activity.

It has been shown that hypoxia increases GABA concentration in brain tissues, depending on severity and duration of hypoxia (Wood et al. 1968; Iversen et al. 1983). Furthermore, the depletion of ATP due to severe hypoxia causes an increase in the levels of GABA and its precursor glutamate in the brain (Madl et al. 2000). While ventilation increases in acute hypoxia, the ventilatory response to acute sustained hypoxia is biphasic. Initial hyperventilation phase is followed by ventilatory depression which is also called "roll-off" (Bureau et al. 1984; Vizek et al. 1987; Karaturan et al. 1996). Acute sustained hypoxia is believed to change the release and turnover of neurotransmitters such as glutamate, GABA, dopamine, adenosine, and serotonin in the brain (Richter et al. 1999). Therefore, the balance between inhibitory and excitatory neurotransmitters

are disrupted and respiratory-related neurons are depressed. However, the underlying mechanism of this biphasic response is unclear. Our previous studies showed that central dopaminergic pathways played an important role in the occurrence of acute hypoxic ventilatory depression (Güner et al. 2002) and that adenosine antagonist (theophylline) prevents the hypoxic ventilatory decline (Karaturan et al. 1996). The prevention of "roll-off" by GABA antagonists (Melton et al. 1990), the increase of GABA concentration during acute hypoxia (Wood et al. 1968; Iversen et al. 1983; Madl et al. 2000), and the observation of an increase in GABA along with glutamate in extracellular fluid of ventral respiratory group of neurons in the initial hyperventilation phase of acute sustained hypoxia (Richter et al. 1999) make this subject still more interesting. Thus, there is a controversy on whether GABA has a role in the "initial hyperventilation phase" or in the "ventilatory depression phase" of the biphasic response.

In the present study, we aimed to investigate whether GABA had an effect on the occurrence of central ventilatory depression seen in acute hypoxia in animals whose peripheral chemoreceptors were denervated. We administered GABA directly using the intracerebroventricular route to investigate:

a- whether GABA induces respiratory depression in rabbits with intact and denervated peripheral chemoreceptors in normoxia.

b- whether GABA affects respiratory responses to acute hypoxia in rabbits with intact and denervated peripheral chemoreceptors.

c- whether inhibitory GABAergic or excitatory glutamatergic mechanisms are functional in response to acute hypoxia when GABA and glutamate antagonists are used in rabbits with intact and denervated peripheral chemoreceptors.

MATERIALS AND METHODS

Experiments were carried out on twenty albino rabbits with a mean weight of 2.5 ± 0.3 kg. Animals were anesthetized with Na-

pentobarbitone (initial dose, 25 mg/kg i.v. thereafter 5 mg/kg/h i.v.) to produce a steady level of anesthesia. The level of anesthesia was determined that animals had reached a surgical plane of anesthesia when they became flaccid and no longer exhibited either an eye-blink or limb-withdrawal. Supplementary doses of anaesthetic were given when mean arterial blood pressure increased above the initial value, the respiratory frequency above 60 min^{-1} , and when the irregular fluctuations occurred in heart rate. Animals were killed by i.v. injection of an overdose (500 mg/kg) of sodium pentobarbitone that caused rapid and irreversible cardiac arrest. Our protocol and methods were approved by the Animal Care and Use Committee of the Laboratory Animal Service of the University of Istanbul, Turkey. Tracheotomy was performed and the tracheal cannula connected to an inspiratory-expiratory valve was inserted into the trachea. The tidal volume (V_T) and respiratory frequency (f_R) were recorded on a polygraph (Grass Model 7, West Warwick, RI, USA) by means of a pneumatograph and a Grass PT-5 volumetric pressure transducer. Tidal volume was measured by way of integrated air flow from expiratory outlet of the valve. From these parameters, respiratory minute volume (\dot{V}_E) was calculated. The right vena jugularis and right arteria femoralis were isolated. All rabbits were given heparin (500 U/kg i.v.) in order to prevent thrombosis which could occur before the experiments.

Denervation of peripheral chemoreceptors

In order to denervate the carotid chemoreceptors, bifurcation regions of arteria carotis communis were isolated bilaterally. N. caroticus were cut. These regions were firstly flushed with phenol and then with alcohol, after which the sites were rinsed thoroughly with physiologic serum. To denervate the aortic chemoreceptors, N. aorticus were isolated and cut bilaterally in the middle cervical region. Chemodenervation was tested by the absence of respiratory response to NaCN ($40 \mu\text{g/kg}$ i.v.) injection.

ICV catheter placement

A cannula was inserted intracerebroventricularly (ICV) to the left lateral ventricle with stereotaxic method. Skulls of the animals were fixed to the stereotaxy device (Stoelting Co., Stellar Cat. No: 51400, Wood Dale IL, USA), after which the scalps were incised at eye-level and the periosteum covering the bone was peeled. Skull was penetrated at 13 mm anterior to Lambda and at 2.5 mm to the left of the midline with a dentist's drill without damaging the duramater. A screw was placed (to the point approximately 2 mm away from the hole) into the skull in order to fixate the cannula after placement. The tip of the cannula fixed to the stereotaxy device was positioned opposite the hole in the skull. The cannula was placed in the left lateral ventricle at 9 mm depth and at the angle of 90° . Acrylic cement (Croform acrylic powder+cold liquid) was used to fixate the cannula.

Drugs

Central administrations of substances were made through the ICV catheter placed in the left lateral ventricle, using a Hamilton injector. The concentration of ICV GABA (Sigma) administration was determined by an assessment of the former studies which had used GABAergic agonists (Soto-Arape et al. 1995) and antagonists (Melton et al. 1990), and by the help of dose-response curves. The dose of GABA, which caused respiratory depression but which did not give rise to apnea, was determined as 0.48 mg/kg . GABA_A antagonist bicuculline was obtained in crystalline form from Sigma Chemical Company. NMDA antagonist, MK-801 was obtained in crystalline form from Merck Pharmaceuticals. The doses of bicuculline and MK-801 were performed as 0.2 mg/kg and 0.09 mg/kg respectively. These doses were selected on bases of earlier studies that showed complete antagonism of GABA and glutamate effects of the respiratory responses from the central nervous system (Yamada et al. 1981; Ang et al. 1992). These agents were dissolved in physiologic serum. All injections were performed

in 0.1 ml volume. All solutions were freshly prepared on the study day.

Isolation of phrenic nerve

Phrenic nerve was carefully isolated on the right side of the cervical regions by a stereomicroscope. The nerve was severed at the 1/3 lower part of the cervical region, at the level above which branches from the 5th and 6th nerve roots interconnect. The sheath of a portion of phrenic nerve coming from the 5th root was bared under microscope, after which the nerve was placed on bipolar platinum electrode to record nerve action potentials. This region was held under neutral paraffin in order to preserve the activity of the nerve during the test procedure.

Experimental procedure

The test animals were divided into two groups. Intact group: rabbits with intact peripheral chemoreceptors (10); Chemodenervated group: rabbits with denervated peripheral chemoreceptors (10). Tidal volume (V_T), respiratory frequency (f_R /min.), systemic arterial blood pressure, and integrated phrenic nerve activity (IP_A) which shows both the amplitude and frequency of phrenic nerve action potentials were recorded with a polygraph (Grass Model 7, West Warwick, RI, USA) at indicated experiment phases in the control and chemodenervated rabbits. Expired ventilation (\dot{V}_E) and mean systemic arterial pressure (BP) were calculated by the recorded parameters. Arterial blood samples obtained at each experiment phase were taken and PaO_2 , $PaCO_2$, and pHa values were measured with an instrument for blood gas analysis (Blood Gas Ciba Corning 860). At the beginning of the experimental phase, when the animals were allowed to breathe air and hypoxic gas mixture (8% O_2 -92% N_2), respiratory parameters and systemic arterial pressure in the both groups were determined. Then, GABA (0.48 mg/kg) was injected through the ICV catheter in one minute while the animal breathed room air and the indicated parameters were recorded. After the respiratory parameters and BP returned

to the normal levels (in 30 minutes), which were measured before GABA administration, the same concentration of ICV GABA was administered in one minute. Then the test animals were breathed hypoxic gas mixture (8% O_2 -92% N_2). GABA_A antagonist (bicuculline, 0.2 mg/kg) and glutamate n-methyl-d-aspartate (NMDA) antagonist, (MK-801, 0.09 mg/kg) were used to determine the reason for the hypoxic response in both experimental groups after GABA administration. After GABA+hypoxic phase, bicuculline was administered and hypoxic phase was applied to the test animals following air phase. Then, animals were allowed to breathe air (normoxia) for 30 minutes until respiratory parameters and BP return to normal levels. After GABA+hypoxia phase and air phase, MK-801 was administered and animals were allowed to breathe hypoxic gas mixture. After the experiment, 1/10 N HCl was administered through a ICV catheter in order to confirm that the catheter was actually at the right localization during the experiment. Methylene blue was then administered through the catheter, after which craniotomy was done. Cerebrum was divided at the midline and methylene blue was seen in the ventricle.

Statistical analysis

Analysis of statistical significance of differences in V_T , f_R , \dot{V}_E , BP, PaO_2 , $PaCO_2$, and pHa before and after ICV injection of GABA, GABA_A, and glutamate antagonists (bicuculline and MK-801) during normoxia and hypoxia was performed with the Wilcoxon-Matched Pairs test. In all phases, $p < 0.05$ was considered significant.

RESULTS

Intact group

Hypoxia. Significant increases in f_R , V_T , and \dot{V}_E were detected while intact animals were breathed hypoxic gas mixture (8% O_2 , 92% N_2) for 3 minutes ($p < 0.05$, $p < 0.01$, $p < 0.01$). Integrated phrenic nerve activity (IP_A), which reflects the activation status of the respiratory centers, increased significantly during hypoxia

($p < 0.01$) (Table 1). Mean systemic arterial blood pressure (BP) values also showed a significant increase in this phase ($p < 0.01$) (Table 2).

ICV GABA. As expected, significant decreases in f_R , V_T , \dot{V}_E and IP_A values were observed in the intact group of rabbits after the first few minutes of ICV GABA (0.48 mg/kg) administration ($p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.01$, respectively) (Table 1). The decrease in BP was found to be significant ($p < 0.01$) (Table 2).

ICV GABA+Hypoxia. Following the administration of the ICV GABA (0.48 mg/kg), when the intact animals were allowed to breathe hypoxic gas mixture, significant increases in f_R , V_T , and \dot{V}_E were obtained ($p < 0.01$, $p < 0.05$, $p < 0.01$, respectively). Meanwhile, a similar increase in IP_A , reflecting the activation of respiratory centers ($p < 0.01$) supports our finding (Table 1). Significant increases in BP values were also detected in this phase ($p < 0.05$) (Table 2).

Following GABA

Bicuculline+Hypoxia. To determine the reason for the hypoxic hyperventilation seen in the intact group in spite of GABA administration, ICV bicuculline (GABA_A antagonist) (0.2 mg/kg) was administered following GABA administration. After bicuculline administration, during hypoxic gas mixture (8% O₂-92% N₂) breathing f_R , V_T , \dot{V}_E , IP_A , and BP significantly increased ($p < 0.001$, $p < 0.01$, $p < 0.001$, $p < 0.001$, $p < 0.001$, respectively) (Tables 1 and 2). The responses of the indicated parameters were higher than those in ICV GABA+ Hypoxia.

MK-801+Hypoxia. As indicated above, bicuculline did not prevent the hypoxic hyperventilation in rabbits with intact peripheral chemoreceptors. This is why we attempted to test whether glutamate antagonist prevented the hypoxic hyperventilation. Following glutamate NMDA antagonist, MK-801 (0.09 mg/kg), when the animals were allowed to breathe hypoxic gas mixture (8% O₂-92% N₂), no significant responses in f_R , V_T , \dot{V}_E , IP_A were obtained (Table 1). Hypoxic hyperventilation seen despite GABA administration

was completely abolished. On the other hand, the response of BP was found to be significantly decreased (Table 2).

Chemodenervated group

Hypoxia. As it can be seen from Table 1 during hypoxic gas mixture (8% O₂-92% N₂) breathing by the peripheral chemoreceptors-denervated rabbits, f_R , V_T , and \dot{V}_E decreased significantly as a result of the depressor effect of hypoxia on the respiratory centers ($p < 0.01$, $p < 0.01$, $p < 0.01$) respectively (Fig. 1, Table 1). The suppression of IP_A in this phase ($p < 0.01$) shows the presence of a hypoxic depression (Fig. 1, Table 1). The decrease in BP values reflects a suppression of cardiovascular mechanisms in the absence of peripheral chemoreceptors (Fig. 1, Table 2).

ICV GABA. ICV GABA administration (0.48 mg/kg) to peripheral chemoreceptors denervated rabbits also did not result in a significant change in f_R , whereas it caused a decrease in V_T and \dot{V}_E ($p < 0.05$, $p < 0.05$) (Table 1). The decrease in IP_A was compatible with the decrease in respiratory parameters ($p < 0.05$). ICV GABA administration (10.48 mg/kg) in this group caused a decrease in respiratory parameters, as well as BP values ($p < 0.01$) (Table 2).

ICV GABA+Hypoxia. After ICV GABA (0.48 mg/kg) administration, no significant change was observed in f_R while the chemodenervated animals were breathing hypoxic gas mixture. But significant increases were obtained in V_T and \dot{V}_E ($p < 0.01$, $p < 0.01$). IP_A also increased significantly in this phase ($p < 0.01$) (Figs. 2A and 3, Table 1). During hypoxic gas mixture breathing by the peripheral chemoreceptors denervated rabbits after administration of ICV GABA, BP was also found to be decreased (Fig. 2A, Table 2).

Following GABA

Bicuculline+Hypoxia. When the chemodenervated group was breathed hypoxic gas mixture, an unexpected increase in respiratory parameters in spite of GABA administration was detected. To determine the reason for the hypoxic hyperventi-

TABLE 1. The mean and standard error (Mean±S.E.) values of f_R , V_T , \dot{V}_E and IP_A of the peripheral chemoreceptors intact and denervated groups of rabbits in the indicated experimental phases

Experimental Phase (n=10)	f_R (min ⁻¹)		V_T (ml)		\dot{V}_E (ml min ⁻¹)		IP_A (mV)	
	Control	Change	Control	Change	Control	Change	Control	Change (%)
<i>Intact</i>								
Hypoxia	63.2±12.7	68.2±14.3*	24.5±15.3	29.6 ±15.6**	1501.5±900.7	1898.1±926.8**	0.46±0.07	+38.9±0.12**
GABA+Air	63.7±15.2	53.6±16.1*	25.9±14.7	19.9±6.4**	1647.6±984.9	1090.0±518.5**	0.48±0.09	-51.8±0.09**
GABA+Hypoxia	54.9±14.8	67.4±15.6**	21.9±10.0	25.5±10.1*	1269.3±537.7	1680.7±461.3**	0.47±0.13	+32.0±0.12**
<i>Following GABA</i>								
Bicuculline+Hypoxia	54.3±13.4	78.4±12.5***	18.5±7.8	29.6±9.7**	1040.7±764.6	2265.8±445.8**	0.25±0.11	+180.0±0.15***
MK-801+Hypoxia	55.6±8.6	58.0±10.8	20.2±5.9	19.0±6.7	1115.1±612.6	1105.8±505.8	0.26±0.08	-4.0±0.11
<i>Chemodenervated</i>								
Hypoxia	58.3±8.9	51.4±8.7**	20.2±6.1	14.2±6.0**	1203.9±411.3	718.0±288.7**	0.41±0.23	-13.9±0.08**
GABA+Air	58.0±15.2	56.4±13.5	21.0±2.9	15.2±3.0*	1263.0±362.0	840.0±273.0*	0.40±0.12	-14.8±0.06*
GABA+Hypoxia	57.2±13.2	58.3±12.6	18.0±3.2	22.6±4.1**	1009.6±287.4	1350.8±312.3**	0.39±0.04	+25.5±0.15**
<i>Following GABA</i>								
Bicuculline+Hypoxia	55.2±10.6	57.5±16.6	17.5±3.6	24.5±4.3**	935.6±376.5	1390.7±425.8**	0.32±0.09	+55.2±0.08**
MK-801+Hypoxia	54.5±18.2	45.0±11.9**	17.0±2.8	14.5±5.2*	912.0±404.5	705.9±208.2**	0.31±0.15	-9.5±0.04*

f_R , respiratory frequency; V_T , tidal volume; \dot{V}_E , expired ventilation; IP_A , integrated phrenic activity; hypoxia, 8% O₂-92%N₂.
*p<0.05, **p<0.01, ***p<0.001 for change compared with control (beginning value).

TABLE 2. The mean and standard error (Mean±s.e.) values of PaO₂, PaCO₂, pH_a and BP of the peripheral chemoreceptors intact and denervated groups of rabbits in the indicated experimental phases

Experimental Phase (n=10)	PaO ₂ (mmHg)		PaCO ₂ (mmHg)		pH _a		BP (mmHg)	
	Control	Change	Control	Change	Control	Change	Control	Change
<i>Intact</i>								
Hypoxia	87.2±4.1	39.2±4.6***	39.0±3.8	35.5±4.2**	7.34±0.05	7.37±0.04**	94.7±9.0	109.0±8.0**
GABA+Air	86.3±5.6	65.8±4.3**	38.0±4.8	41.3±2.8*	7.35±0.05	7.33±0.01*	98.4±8.0	69.0±11.9**
GABA+Hypoxia	88.3±5.2	40.2±3.6***	38.7±3.2	34.6±1.6**	7.35±0.03	7.38±0.04*	85.0±19.9	100.2±15.0*
<i>Following GABA</i>								
Bicuculline+Hypoxia	66.6±4.7	39.6±3.9**	39.1±2.3	35.0±3.3**	7.34±0.04	7.37±0.03**	75.0±9.2	127.6±12.5***
MK-801+Hypoxia	68.4±3.6	40.3±2.9**	37.8±3.1	37.5±4.6	7.35±0.02	7.36±0.01	70.0±16.5	50.5±9.0**
<i>Chemodenervated</i>								
Hypoxia	79.6±2.8	40.9±5.4**	39.5±1.8	42.9±4.3**	7.35±0.03	7.32±0.01	116.5±27.1	93.2±23.8**
GABA+Air	81.3±3.4	66.1±2.5**	40.2±3.1	42.8±3.8*	7.35±0.01	7.32±0.03*	124.2±17.5	95.5±22.5**
GABA+Hypoxia	80.6±3.5	41.2±1.7***	40.1±4.1	38.5±3.2*	7.34±0.04	7.36±0.05*	111.5±23.0	75.5±15.2**
<i>Following GABA</i>								
Bicuculline+Hypoxia	65.3±4.2	39.2±2.1	39.9±2.6	37.8±2.4*	7.34±0.02	7.36±0.05*	85.8±17.3	135.9±22.5***
MK-801+Hypoxia	65.7±3.9	39.0±3.5	40.0±2.7	42.3±1.9**	7.33±0.05	7.3±0.05**	90.7±19.2	55.0±23.4***

BP, mean systemic arterial pressure.

p*<0.05, *p*<0.01, ****p*<0.001 for change compared with control (beginning value).

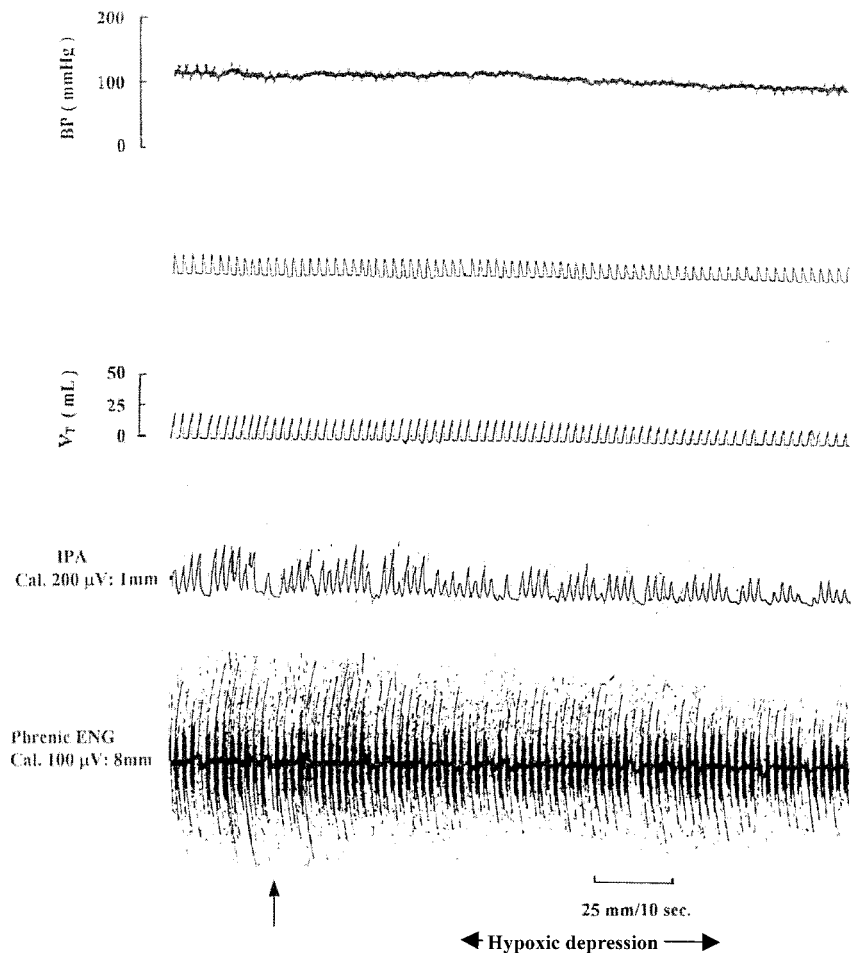


Fig. 1. Responses to hypoxia in chemodenervated group. Hypoxia: 8% O₂-92% N₂. Traces from top to bottom are systemic arterial blood pressure (BP), tidal airflow, tidal volume (V_T), integrated phrenic activity (IP_A), phrenic electroneurogram (ENG) respectively. The arrow indicates the beginning of the hypoxic phase. The decreases in V_T and IP_A show hypoxic depression. To see the phrenic activity clearly, the paper rate of the polygraph was increased. For this reason the end of the hypoxic phase could not indicated in the figure.

lation, ICV bicuculline (0.2 mg/kg), the GABA_A antagonist, was administered and hypoxic phase was repeated. During hypoxic gas mixture (8% O₂-92% N₂) breathing after bicuculline administration, V_T, \dot{V}_E , IP_A, and BP significantly increased ($p < 0.01$, $p < 0.01$, $p < 0.01$, $p < 0.001$, respectively) (Figs. 2B and 3, Tables 1 and 2). There was no significant change in f_R (Fig. 3, Table 1).

MK-801+Hypoxia. As shown above, because bicuculline did not eliminate the hypoxic hyperventilation in the chemodenervated rabbits as in the intact group, glutamate NMDA antago-

nist (MK-801) (0.09 mg/kg) was used to investigate the reason for this response. Following glutamate antagonist, MK-801, when the chemodenervated animals were allowed to breathe hypoxic gas mixture (8% O₂-92% N₂), significant decreases in f_R , V_T, \dot{V}_E , and IP_A were obtained ($p < 0.01$, $p < 0.01$, $p < 0.05$, $p < 0.05$, respectively) (Figs. 2C and 3, Table 1). Hypoxic hyperventilation seen despite GABA administration was completely abolished as in the intact group. There was a significant decrease in BP ($p < 0.001$) (Fig. 2C, Table 2).

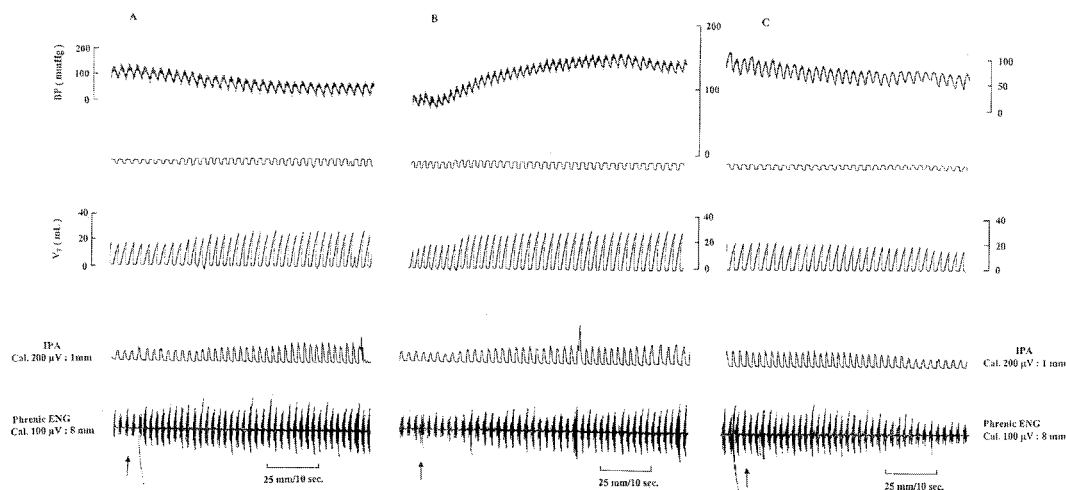


Fig. 2. Responses to GABA (0.48 mg/kg)+Hypoxia (A), Bicuculline (0.2 mg/kg)+Hypoxia (B) and MK-801 (0.09 mg/kg)+Hypoxia (C) in peripheral chemoreceptors denervated rabbits (chemodenervated group). Hypoxia: 8% O₂-92% N₂. Traces from top to bottom are systemic arterial blood pressure (BP), tidal airflow, tidal volume (V_T), integrated phrenic activity (IP_A), phrenic ENG respectively. The arrow indicate the beginning of the experimental phase. To see the phrenic activity clearly, the paper rate of the polygraph was increased. For this reason the end of the hypoxic phase could not indicated in the figure.

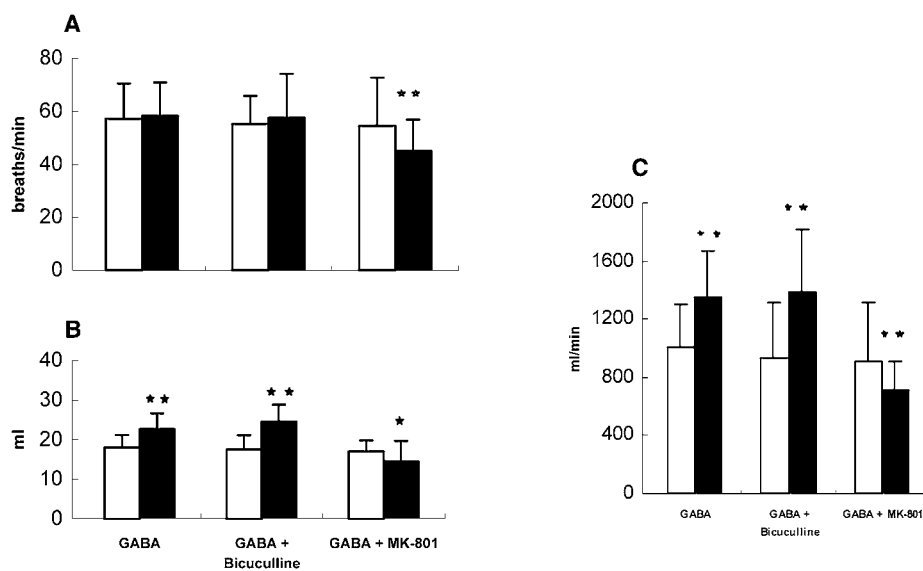


Fig. 3. The changes of respiratory frequency (f_R), tidal volume (V_T) and expired ventilation (V_E) in GABA+Hypoxia, Bicuculline+Hypoxia and MK-801+Hypoxia in chemodenervated group. Hypoxia: 8% O₂-92% N₂. A: f_R , B: V_T , C: V_E . □, Air; ■, Hypoxia.

As shown in Table 2, variations in PaO₂, PaCO₂, and pH_a values reflected the changes of the respiratory parameters during each experimental phase.

DISCUSSION

In the present study, the increases in respiratory parameters (f_R , V_T , \dot{V}_E) and integrated phrenic activity (IP_A) of the animals with intact peripheral chemoreceptors during hypoxic gas mixture breathing were mainly due to the stimulation of peripheral chemoreceptors (Oruç et al. 1982; Şahin et al. 1985). Acute hypoxia affects neural activity and amino acid metabolism in the same way. Recent studies suggested that afferent inputs from peripheral chemoreceptors probably lead to the release of glutamate in nucleus tractus solitarius and increase excitatory drive by activating AMPA and NMDA receptors in respiratory neurons (Kalia 1981; Lawson et al. 1989; Ang et al. 1992; Mizusawa et al. 1994). Inhibition of glutamate with specific antagonists almost totally abolishes the hyperventilatory response to hypoxia (Soto-Arape et al. 1995). On the other hand, it was shown that glutamate and GABA concentrations increase simultaneously in the extracellular fluid of ventral medullary respiratory group during the hyperventilation observed in the first few minutes of hypoxia (Hoop et al. 1999; Richter et al. 1999). As it is well known, under normal conditions GABA is synthesized from glutamate by the effect of GAD, and the extracellular concentration of GABA is kept very low by GABA transporters. In addition, ATP depletion induced by hypoxia may increase GAD activity. During ATP depletion induced by hypoxia, glutamate concentrations remain high in terminals, leading to an increase in GABA synthesis (Madl et al. 2000). Hypoxia may also increase GABA levels by inhibiting GABA metabolism by GABA transaminase due to the low pH induced by hypoxia/ischemia (Madl et al. 2000).

These studies (Hoop et al. 1999; Richter et al. 1999) suggested that simultaneous increases in both glutamate and GABA during hypoxia may

also play an important role in acute sustained hypoxia and in the occurrence of hypoxic ventilatory depression (HVD). The disruption of the balance between excitator and inhibitor neurotransmitters may affect the response for respiratory-related neurons.

ICV GABA administration decreased ventilation by its inhibitory effect on central respiratory neurons in spite of the impulses originating from the peripheral chemoreceptors during normoxia. As it is well known, peripheral chemoreceptors are active during normoxia and the facilitatory inputs from peripheral chemoreceptors burst central inspiratory activity (CIA) (Oruç et al. 1982; Vizek et al. 1987). GABA produces this inhibitor effect by causing hyperpolarization due to an increase in the influx of Cl⁻ into the cardiorespiratory neurons (Anderson et al. 1980; Neubauer et al. 1990). Under normoxic conditions, centrally administered exogenous GABA or GABA analogues depress ventilation (Yamada et al. 1981, 1982; Kneussl et al. 1986; Taveira-Da Silva et al. 1987), whereas GABA antagonists bicuculline and baclofene cause an increase in phrenic activity (Taveira-Da Silva et al. 1987; Melton et al. 1990; Soto-Arape et al. 1995). These findings suggest that GABAergic mechanisms are tonically effective in the regulation of respiratory activity under normoxic conditions. We found that respiration reduced after ICV GABA administration during normoxia. These findings are in line with those of others.

After ICV GABA administration, the significant increase in ventilation during hypoxic gas mixture breathing was an unexpected finding for us. Central GABA administration did not prevent the excitatory effects of increased peripheral chemoreceptor impulses on central respiratory neurons during hypoxia. On the other hand, glutamate and other excitatory agents, which are present in increased amounts in the brain as a result of hypoxia, may overcome the effects of exogenous GABA. One reason for that is, hypoxia may derange uptake and enzyme activities in the neurons (Neubauer et al. 1990; Haddad and Jiang

1993). The increase in exogenous GABA may prevent the rise in endogenous GABA during hypoxia. The increase in the GABA content of the brain may prevent the conversion of glutamate to GABA by inhibiting GAD activity, thereby causing an increase in glutamate. Excitatory and inhibitory synaptic connections both have a role in the regulation of the respiratory rhythm (Richter et al. 1999). It is known that reciprocal inhibitory synapses are a characteristic of the respiratory circuit. Administration of GABA along with hypoxic stimulation may produce a balance between inhibitory and excitatory neurotransmitters, however it does not cause a disruption in the hypoxic response, as in the present study. The increase in glutamate and GABA in the phase in which ventilation increases during hypoxia (Hoop et al. 1999; Richter et al. 1999) and the increase in GABA in vivo and in vitro in hypoxia (Wood et al. 1968; Iversen et al. 1983; Madl et al. 2000) also support this unexpected finding. Thus, the hypothesis that glutamate is solely responsible for the increase in acute sustained hypoxic response while GABA is responsible of acute sustained hypoxic depression (Chiang et al. 1986; McCrimmon et al. 1989; Kazemi and Hoop 1991) seems to weaken.

In the rabbits with denervated peripheral chemoreceptors, acute hypoxia gave rise to respiratory depression, as expected. As it is well known, peripheral chemoreceptors are essential in the hypoxic ventilatory response. Chemodenervation abolishes the hypoxic hyperventilatory response. Hence, hypoxia depresses ventilation following chemodenervation in many species (Robbins 1995; Weil 1999). On the other hand, the oxygenation of the respiratory centers plays an important role in the regulation of V_T in the absence of impulses originating from the peripheral chemoreceptors during hypoxia (Oruç et al. 1982).

After ICV GABA administration, when the peripheral chemoreceptors-denervated animals were allowed to breathe hypoxic gas mixture, increased ventilation was another unexpected finding. In our experiment, GABA was injected into the lateral ventricle. This administration method

may suppress not only the medulla oblongata but also higher brainstem and other brain regions. It was reported that supramedullary brain regions are involved in hypoxic ventilatory depression (Monin et al. 1999) and send inhibitory signals to the medulla oblongata during hypoxic exposure due to release and local accumulation of inhibitory neurotransmitters (GABA, dopamine, adenosine). Under these conditions, injection of GABA into the lateral ventricle may suppress hypoxia-induced ventilatory inhibitory mechanisms of the midbrain and pons; this suppression may reverse hypoxic ventilatory depression and even increase ventilation during hypoxia. But in our experiment, the hypoxic hyperventilation seen after GABA administration occur not only in peripheral chemoreceptors intact animals, but also in peripheral chemoreceptors-denervated animals (Fig. 3). Therefore, the increased ventilation following exogenous ICV GABA administration may be attributed either to GABA-evoked block of the cerebellar inhibitory input during hypoxia (Monin et al. 1999) or to the releasing of the glutamate by the effect of both exogenous GABA and hypoxia, independent of the stimulation of peripheral chemoreceptor afferents. On the other hand, the response of BP was found to be significantly decreased, as expected. The depression in BP in this phase shows again the importance of the impulses from the peripheral chemoreceptors on cardiovascular regulation.

In the present study, in order to identify better underlying mechanism of the increased hypoxic ventilatory responses in spite of GABA administration in the intact and peripheral chemoreceptors-denervated rabbits, the antagonists, GABA_A (bicuculline) and glutamate NMDA (MK-801) were used. Following GABA administration, when intact and peripheral chemodennervated animals were breathed hypoxic gas mixture after ICV bicuculline administration, both V_T and IP_A increased. Bicuculline is a well-studied antagonist of the GABA_A receptor, which is linked to chloride gating. It decreases chloride flux, resulting in depolarization. These findings show hy-

poxia-induced ventilatory inhibitor mechanisms are prevented because GABA administration in to the lateral ventricle is not responsible for the hypoxic hyperventilation in our experimental protocol. Furthermore, we did not observe a reduction in V_T due to bicuculline administration during hypoxia in both groups (Fig. 3). On the other hand, the increase in the GABA content of the brain in hypoxia may prevent the conversion of glutamate to GABA by inhibiting GAD activity, thereby causing an increase in glutamate. One can speculate that GABA may influence respiration during hypoxia indirectly via other transmitter systems as it takes place in the neuronal network of the central nervous system. It was also suggested that the increased activity seen in hypoxia, at least partly, is also due to the increased release of glutamate by directly activating the ventral respiratory neuron circuits, since early hypoxic increases in respiratory discharges also occur in peripheral chemodenervated animals (Richter et al. 1991). For this reason, the increase in ventilation with acute hypoxia in both groups in spite of GABA administration is thought to be mediated with such excitatory neurotransmitters as glutamate. In our study, when ICV glutamate antagonist (MK-801) was administered, the hypoxic hyperventilatory responses seen in both groups were abolished. It was shown that acute hypoxia increases glutamate turnover (Hoop et al. 1999; Richter et al. 1999). Respiratory neurons receive glutamergic tonic inputs, which activate both NMDA and AMPA receptors (Haji et al. 2000). Furthermore, it was suggested that short-term potentiation is mediated by NMDA receptors, which augment EPSPs and prolong depolarization of phrenic motoneurons (McCrimmon et al. 1997). NMDA mechanisms are also responsible for phase transition from inspiration to expiration. Thus, exogenous GABA may increase ventilation by increasing the level of glutamate in the brain, independent of peripheral chemoreceptors in hypoxia as shown in Fig. 3.

The responses of BP after bicuculline and MK-801 administrations show that cardiovascular mechanisms are under both GABAergic and glu-

tamatergic control during hypoxia.

In conclusion, our findings suggest that ICV GABA injection suppresses cardiorespiratory centers both in the presence and absence of peripheral chemoreceptors in accordance with its inhibitory characteristics in normoxic conditions. In contrast, ICV GABA causes an increase in ventilation during hypoxic conditions with or without peripheral chemoreceptor impulses by increasing glutamate which may be synthesized or released by the effect of GABA via a mechanism not clearly understood. For this reason, our results suggest that exogenous GABA can not be held responsible for acute hypoxic ventilatory depression. On the contrary, both GABA and glutamate may be responsible for acute hypoxic hyperventilation.

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