Hypoxic Initiation of Pulmonary Hypertension is Mediated by Serotonin Secretion from Neuroepithelial Bodies in Chemodenervated Dogs

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Abstract

The purpose of this study was to investigate the stimulatory effect of hypoxia on the secretion of serotonin by neuroepithelial bodies (NEB) as well as to determine the relation between its level and changes in pulmonary arterial pressure (PAP) and also to determinate the effect of serotonin antagonists (pizotifen and methysergide) on the responses of pulmonary and systemic arterial pressures. The experiments were carried out in peripheral chemoreceptor-denervated dogs anesthetized with Na penthabarbital (30 mg/kg i.v.). On the breathing of normoxic and hypoxic (7% O_2-93% N_2) gas mixtures and on the injection of KCN (80 μg/kg i.v.), PAP, systemic arterial blood pressure (BP), tidal volume (V_T), respiratory frequency (f/min), ventilation minute volume (V_E) were determined. Also PAP and BP were recorded before and after the injection of pizotifen (0.5 mg/kg i.v.) and methysergide (1 mg/kg i.v.) during normoxic or hypoxic gas mixture breathing. At the end of each experimantal phase, serotonin level, PaO_2, $PaCO_2$ and pH_a values in blood samples obtained from left ventricle and femoral artery were determined. On the breathing of the hypoxic gas mixture of the chemodenervated dogs, V_T, V_E and BP significantly decreased ($P<0.001$, $P<0.001$, $P<0.01$). The mean value of PAP and serotonin levels (ventricular and femoral) were found significantly increased when compared with the corresponding normoxic values ($P<0.001$, $P<0.05$). On the other hand, injection of KCN produced no significant changes in PAP, serotonin levels, BP and respiratory parameters. After the injection of pizotifen, PAP was significantly increased in hypoxia ($P< 0.01$). After the injection of methysergide, the response of PAP was completely abolished during the breathing of hypoxic gas mixture. The finding of the abolition of response of PAP to hypoxia after the injection of methysergide indicates that serotonin release from NEB may be responsible for the elevation of PAP in hypoxic hypoxia.

Key Words: neuroepithelial bodies, hypoxia, pulmonary arterial pressure, serotonin, serotonin antagonists, dog

Introduction

Neuroepithelial bodies (NEB), first discovered in Lauweryns' tissue sections in 1971, are structures composed of afferent and efferent nerve endings that are located in bifurcations of large airways and are sensitive to the oxygen partial pressure in the inspired air. There are dense-core vesicles containing various biological amines in their cytoplasms (4, 6).

Ultrastructural studies have shown the presence of two types of dense-core vesicles (DCV_1, DCV_2) in NEB cytoplasm (12, 14). In a study on neonatal rabbits, serotonin was detected only in DCV_1 and no immunologic reactivity was observed in DCV_2 (14).

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Thus, NEB gives the impression of an important source of intra-pulmonary serotonin (4, 6).

The use of in vitro models of isolated NEB combined with electrophysiological studies have shown that NEB cells express an O_2 sensor protein (identified as a multicomponent NADPH oxidase) linked to a O_2-sensitive K^+ current (4, 8, 25). According to the "membrane" model of O_2 sensing hypoxia affects the function of the oxidase, resulting in reduced reactive oxygen species production, including H_2O_2, leading to closure of the O_2-sensitive K^+ channels followed by membrane depolarization, opening of voltage -activated Ca^{2+} channels, influx of extracellular Ca^{2+}, and neurotransmitter release (17).

Serotonin was released by exocytosis of DCV1 from the basement membrane in acute and chronic hypoxia and a decrease in serotonin content of NEB was shown both in vivo and in vitro studies with various histological methods and electron microscopy (4, 12, 15, 16). NEB require an increase in Ca^{2+} for stimulus-secretion coupling (5). Yet the role of 5-HT in NEB cell function is remained to be determined.

Serotonin is a strong pulmonary vasoconstrictor and bronchoconstrictor (4, 6). On the other hand, hypoxia is known to cause pulmonary hypertension. Serotonin, which is released from NEB by hypoxic stimulation, may diffuse into the pulmonary vasculature and may be one of the causes of the pulmonary hypertension occuring in hypoxia.

In the present study, in order to answer the question whether pulmonary hypertension observed in acute hypoxic hypoxia is caused by serotonin, we intended to investigate the responses in pulmonary arterial pressures and systemic arterial pressures during hypoxic gas mixture breathing, after administration of two different kinds serotonin antagonists. Serotonin levels was taken as a criterion of NEB stimulation.

Materials and Methods

Eight mongrel dogs of 17-23 kg. in body weight were used as experimental animals. The animals were anesthetized with Na penthabarbital (30 mg/kg i.v.). Tracheotomy was performed and the tracheal cannula, connected to an inspiratory-expiratory valve, was inserted into the trachea. The right jugular vein and right femoral artery were isolated. All dogs were given 500 U/kg liquemine i.v. before the experiment.

In order to determine whether the stimulation of NEB's by hypoxia contribute to increase in ventilation during hypoxic gas mixture breathing chemodenervation was done to eliminate the chemoreceptor impulses.

Carotid nerves were isolated and severed and the surrounding tissues were damaged, at the bifurcation level of common carotid artery bilaterally, for denervation of peripheral chemoreceptors. These regions were also flushed with alcohol and then phenol, after which the sites were rinsed thoroughly with physiologic serum. For the denervation of the aortic area, the aortic nerve was separated from the vagus nerve just below the point where the superior laryngeal nerve leaves the vagosympathetic trunk and cut. Chemodenervation was tested by the absence of ventilatory response to intravenous injection of potassium cyanide (40 μg/kg i.v.).

A trochar catheter was placed in the left ventricle in order to obtain blood samples. An opticath type catheter (8199. TD 1704H TD Catheter 7F 4L with sleeve) was inserted into the pulmonary artery through jugular vein. The catheter was first connected to the polygraph (Grass 7) via a pressure transducer. The pressure change was observed with the help of the polygraph, while the catheter tip passed from the right jugular vein to the right atrium, after which the catheter was introduced into the ventricle while monitoring the pressure changes, and the baloon at the catheter tip was inflated. First the ventricular pressure was recorded, then the baloon obliterated the initial portion of the pulmonary artery and the WEDGE pressure was recorded as a fall in pressure. Pulmonary arterial pressure was recorded after the baloon was deflated.

In the peripheral chemoreceptor-denervated dogs, the pulmonary arterial pressure (PAP), systemic arterial blood pressure (BP), tidal volume (VT), and respiratory frequency (f/min) were recorded with a polygraph during normoxia (air breathing), hypoxic hypoxia and histotoxic hypoxia. Respiratory minute volume (V_E) was calculated from respiratory parameters recorded.

Hypoxic hypoxia was created by hypoxic gas mixture ($7\%O_2$-$93\%N_2$) breathing. Histotoxic hypoxia was produced by administration of KCN (80 μg/kg i.v.) in order to determine whether the NEB's are stimulated by histotoxic hypoxia. For this purpose the dose which stimulates the peripheral chemoreceptors was doubled. KCN (80 μg/kg i.v.) in 3 ml of saline solution was injected by constant infusion over 3 minutes of time during air breathing. After serotonin antagonists pizotifen (0.5 mg/kg i.v.) ($5HT_{1c}$ and $5HT_2$) and methysergide (1 mg/kg i.v.) ($5HT_1$ and $5HT_2$) were injected into the animals in this order the same experimental procedures were repeated.

In each phase of the experiment, blood samples were taken from the catheters in the left ventricule and femoral artery for measurement of serotonin levels by "microcolon chromatography techniques" (3, 23). Also, PaO_2, $PaCO_2$ and pHa values were determined in ventricular and femoral arterial blood samples taken in each phase of the experiment using AVL gas

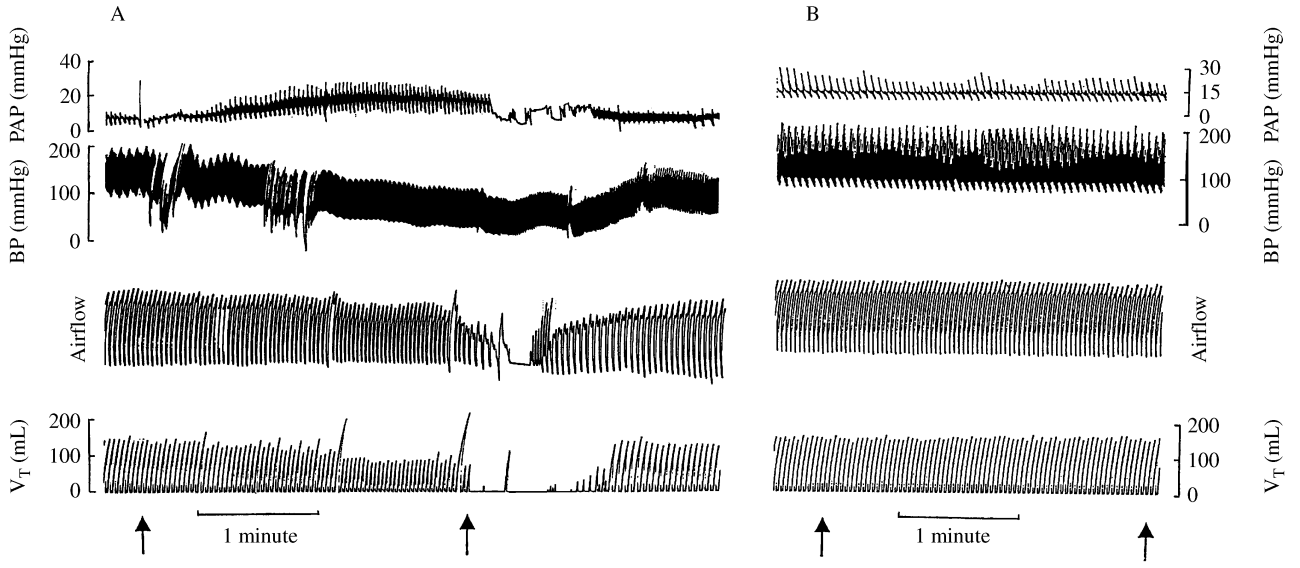

Fig 1. The responses to hypoxic gas mixture breathing (7 % 0_2 - 93% N_2) (A) and administration of KCN (80 μ/kg i.v.) (B) of the peripheral chemoreceptors - denervated dog. When the animal was challenged to hypoxic gas mixture (A), an increase in PAP and a decrease in systemic BP were evoked. Concomitantly, a gradual decrease in V_T and apnea were observed. The arrows indicate the begining and end of the experimental phases.

check type 937, at a temperature of 37°C.

Statistics

The statistical significance of the changes in the PAP, respiratory parameters, BP, serotonin levels and blood gases measured during normoxic and hypoxic phases and before and after KCN injections and before and after injection of serotonin antagonists were tested with Wilcoxon-Matched Paired test. Before and after injection of serotonin antagonists during normoxic and hypoxic phases, statistical analysis were also done.

Results

Changes in Respiratory Parameters

As it can be seen from Table 1, on the breathing of hypoxic gas mixture (7% O_2-93% N_2) by the peripheral chemoreceptors-denervated animals, the respiratory frequency did not change significantly in comparison to the normoxic phase. On the other hand, tidal volume decreased significantly as a result of the depressor effect of hypoxia on the respiratory centers of peripheral chemoreceptors-denervated animals followed by an involuntary apnea (P<0.001). Respiratory minute volume also decreased significantly, as a result of the decrease in tidal volume (P<0.01) (Fig. 1).

Histotoxic hypoxia produced by KCN injection caused no significant changes in f, V_T and V_E of the peripheral chemodenervated animals (Table 1, Fig. 1). As the peripheral chemoreceptors are denervated, histotoxic hypoxia can not stimulate these receptors and no change occurs in the respiratory parameters.

Changes in Mean Pulmonary Arterial Pressures

Changes in mean pulmonary arterial pressures (PAP) in hypoxic hypoxia and histotoxic hypoxia created with KCN injection are shown in Table 1 and Fig. 1. As is to be expected, on the breathing of hypoxic gas mixture by the peripheral chemoreceptors-denervated animals, the mean pulmonary arterial blood pressure was significantly higher than that of the normoxic phase (P<0.001).

On the other hand, no significant change in mean pulmonary arterial blood pressure was observed after KCN injection.

Changes in Serotonin Levels

Changes in serotonin levels measured in ventricular and femoral arterial blood samples are shown in Table 1. The important point is that, in these experimental phases, ventricular and systemic blood samples were taken to measure serotonin levels when a change in PAP was observed.

When the peripheral chemoreceptors-denervated dogs were allowed to breathe hypoxic gas mixture, serotonin levels, were significantly increased in both ventricular and systemic blood (Table 1). Concomitantly, increase in PAP was also observed.

Table 1. The mean (M) and standard error (SE) values of indicated paramaters of the peripheral chemoreceptors-denervated dogs on the breathing of hypoxic gas mixture (7% O_2 - 93% N_2) and after KCN (80 μ/kg i.v.) injection.

Experimental phase (n=8)	f (min^{-1})	V_T (mL)	V_E (mL/min)	PAP (mmHg)	BP (mmHg)	V. Serotonin (μg/mL)	F. Serotonin (μg/mL)
Air	31.9±13.3	179.5±12.5	5810.0±745.8	13.8±1.1	146.5±2.3	6.51±0.75	12.20±1.98
Hypoxia	31.3±2.5	129.4±13.3***	4138.6±635.3**	19.0±1.6***	115.4±9.9**	10.85±2.1*	27.74±6.02*
Air	19.8±1.7	172.0±13.9	3214.0±331.4	16.0±2.0	14 9.6±3.1	11.32±2.92	18.24±4.57
KCN	19.2±1.3	172.4±13.8	3278.0±290.9	16.8±3.2	150.1±3.2	9.76±2.29	16.58 ±2.75

n: denotes the number of determinations
**P*<0.05, ***P*<0.01, ****P*<0.001, indicates statistical significance of the difference between the mean values of the indicated paramaters in the indicated experimental phases. f min^{-1}, respiratory frequency; V_T, tidal volume; V_E, respiratory minute volume; PAP, pulmonary arterial blood pressure and venticular, femoral serotonin levels.

Table 2. The mean (M) and standart error (SE) values of Pa O_2, Pa CO_2, pHa in ventricular and systemic arterial blood samples of peripheral chemoreceptors-denervated dogs in the indicated experimental phases.

Experimental phase (n=8)	PaO_2 (mmHg)		$PaCO_2$ (mmHg)		pHa	
	Ventricular	Systemic	Ventricular	Systemic	Ventricular	Systemic
Air	76.9±6.45	78.9±5.2	43.9±2.1	44.5±2.4	7.42±0.02	7.41±0.02
Hypoxia (7%O_2 - 93% N_2)	31.8±0.4***	34.7±0.5***	45.4±2.1*	47.1±0.4**	7.41±0.03	7.40±0.02
Air	76.2±4.1	79.6±5.6	41.5±1.6	42.9±1.5	7.43±0.01	7.42±0.01
KCN (80 μ.kg^{-1} i.v.)	75.9±3.3	81.3±5.2	41.9±2.1	42.6±2.3	7.43±0.02	7.42±0.01

n: denotes the number of determinations
**P*<0.05, ***P*<0.01, ****P*<0.001, indicates statistical significance of the difference between the mean values of the indicated paramaters in the indicated experimental phases.

In contrast, significant changes in PAP and serotonin levels were not observed with histotoxic hypoxia created with KCN injection (Table 1).

Changes in Systemic Arterial Blood Pressure

On the breathing of hypoxic gas mixture by the peripheral chemoreceptor-denervated dogs BP was significantly diminished (*P*<0.01). No appreciable change in BP was noted during histotoxic hypoxia created with KCN injection (Table 1, Fig. 1).

Changes in PaO_2, $PaCO_2$ and pH_a

As shown in Table 2, mean PaO_2 values in systemic and ventricular arterial blood were significantly decreased on the breathing of hypoxic gas mixture, while $PaCO_2$ values in both blood samples were significantly increased. On the breathing of hypoxic gas mixture no significant changes were observed in ventricular and systemic pH.

After iv KCN treatment significant changes in PaO_2, $PaCO_2$ and pH_a values in both systemic and ventricular blood samples were not observed.

Effects of Serotonin Antagonists

In order to determine if serotonin is responsible for the increase in PAP during hypoxic hypoxia two types of serotonin antagonists pizotifen and methysergide were used in this experimental setting. When the experimental animals breathed a hypoxic gas mixture after administration of pizotifen (0.5 mg/

Table 3. The mean (M) and standart error (SE) values of pulmonary arterial presssure (PAP) and systemic arterial pressure (BP) before and after injection of pizotifen (0.5 mg/kg i.v.) and methysergide (1 mg/kg i.v.) of peripheral chemoreceptors-denervated dogs in the indicated experimental phases.

Experimental phase (n=8)	PAP (mmHg)	BP (mmHg)
Air	12.9±1.2	151.2±2.9
Hypoxia	18.8±1.3***	118.4±9.9***
Pizotifen	13.0±0.9	149.2±8.1
Hypoxia	19.6±1.7***	115.7±7.4***
Methysergide	11.4±0.8	148.9±5.9
Hypoxia	11.4±0.8	117.5±9.6**

n: denotes the number of determination
**P<0.01, ***P<0.001, indicates statistical significance of the diference between the mean values of the indicated parameters in the indicated experimental phases.

kg i.v.), significant increase was detected in PAP (P<0.001). BP was significantly diminished (P< 0.001, Table 3).

On the other hand, hypoxic gas mixture breathing after injection of methysergide (1 mg/kg i.v.) produced no change in PAP (Table 3).

Systemic arterial pressure decreased significantly after the injection of methysergide in hypoxia (P<0.01, Table 3).

Discussion

In the present study, no change in f occurred in peripheral chemoreceptors-denervated dogs, but V_T decreased and this was followed by apnea as expected. This was probably caused by the depressive effect of hypoxia on the respiratory centers of chemoreceptors-denervated animals. In a former study (22), we had investigated the effects of hypoxic stimulation of NEB's on the respiratory pattern. By means of a cross circulation technique, the cerebral circulation of the recipient dog was separated from its systemic circulation and was perfused via the vertebral and internal carotid arteries with blood from the common carotid artery of the donor dog. Hence, facilitatory impulses from the NEB's of the recipient are transmitted to the respiratory centers of the recipient as a consequence of which respiratory activity is augmented in conditions when central oxygenation is normal (13, 22). Following bilateral vagotomy, the respiratory responses to hypoxia were diminished. But in conditions when central structures are hypoxic, these facilitatory impulses are prevented due to a decrease in neuronal activity of the respiratory centers. In absence of peripheral chemoreceptor input the respiratory centers are known to be depressed by direct central depressor effect of hypoxia (10, 18). In this present study, as the central structures are in hypoxic conditions, stimuli going from NEB to the respiratory centers cause a decrease in tidal volume instead of increase. No changes in V_T, f and V_E in peripheral chemoreceptors-denervated animals during histotoxic hypoxia caused by KCN (i.v.) injections would prove the completeness of chemoreceptor denervation.

The use of in vitro models of isolated NEB's, combined with electrophysiological approaches, have provided direct evidence that NEB cells express a membrane-bound O_2 sensor and are the transducers of hypoxic stimulus (4). On the other hand, it is hypothesized that NEB's are also airway receptors contributing to the control of bronchomotor and vasomotor tone in response to the chemical composition of the gas present in the airways (4, 6, 9, 19). Although there still is some controversial on this topic, morphometric and cytochemical studies point to NEB's as the source of intrapulmonary serotonin (6, 7, 14). The hypoxic NEB secretory response was neurally controlled possibly by intrapulmonary axon reflexes in sensory nerve fibers (4).

Our findings, an increase in pulmonary arterial pressure and serotonin levels show the presence of a relation between hypoxic stimulation of NEB's and pulmonary hypertension. NEB are a known source of endogenous 5-HT in the lung, and acute hypoxia appears to be the main "physiological" stimulus for 5-HT release. NEB's are postulated to function as airways O_2 sensors by releasing 5HT during hypoxia. NEB's receive afferent and efferent innervation. Their afferent innervation is predominantly by sensory endings of the vagus nerve. The vagal afferents are intracorpuscular nerve endings, the cell bodies of which are located in nodose ganglia. 5HT, in turn causes the excitation of vagal afferent fiber endings (4, 8, 13, 24). Recent studies suggested that $5HT_3$-R in NEB cells may function as an autoreceptor and may potentially be involved in modulation of hypoxic signalling (8).

In this study high levels of serotonin in samples of left ventricular and femoral arterial blood collected simultaneously with the increase in pulmonary arterial pressure during hypoxic gas mixture breathing, suggests the role played by the increase in serotonin, which is an effective vasoconstrictor in pulmonary vasculature.

Our results show that pizotifen which is known to be $5HT_{1C}$ and $5HT_2$ receptor antagonist (2) produces no change in the response of PAP to hypoxic hypoxia. On the other hand methysergide abolishes the increase

in PAP during hypoxic gas mixture breathing. In other words the significant increase in PAP in response hypoxic hypoxia is prevented by serotonin antagonist methysergide.

A recent study of Launay et al. (11) showed that a selective $5HT_{2B}$ receptor agonist (dexfenfluramine) increases the risk of pulmonary hypertension in humans and mice and that pulmonary hypertension is associated with an increase in $5HT_{2B}$ receptor expression in pulmonary arteries. These findings indicate the important role of $5HT_{2B}$ receptors on the development of PAP. In fact, methysergide was proven to be a powerful antagonist of $5HT_{2B}$ receptors (1). On the basis of this knowledge and our finding we can suggest that the pulmonary hypertension we observed in response to hypoxic hypoxia is mediated by serotonin acting on $5HT_{2B}$ receptors.

It is for this reason that when the animals breathed the hypoxic gas mixture after administration $5HT_{2B}$ receptor antagonist methysergide no increase occurred in PAP. This result strongly suggests the role of serotonin released from NEB's in the increase in PAP during hypoxic hypoxia (14). As it is well known, the apical pole of NEB is in juxtaposition with the airway lumen, and basal or vascular pole is in juxtaposition with fenestrated capillaries (4). The presence of a capillary sulcus connecting NEB's, and that this sulcus is separated from the basement membrane by collagen fibres was shown in a study on rabbits (4, 9). This sulcus was hypothetized to originate from pulmonary artery branches and to drain into the pulmonary veins. The reason of the occurrence of venous constriction in serotonin release was explained by this structure (9). Taken together, serotonin may play a role in the mechanism of pulmonary vasoconstriction occuring as a result of hypoxia, by first being released from the NEB basement membrane as a consequence of an hypoxic stimulus, and then diffusing into the airway smooth muscle, bronchial and pulmonary vascular beds (27).

Prevention of pulmonary hypertension by serotonin antagonists in our study, demonstrates that serotonin is responsible for pulmonary vasoconstriction. On the other hand, we do not have definite proof of serotonin release from NEB. It is known that systemic hypoxia and acidosis cause activation of platelets (26). Vasoconstrictors such as serotonin and TxA_2 (thromboxane) are released from activated platelets (20). For this reason, our finding of increased serotonin in hypoxia may be attributed to platelet activation. In a study by Rustagno et al. (20), platelet activation was not found to be increased in patients with chronic obstructive pulmonary disease (COPD), but a local platelet activation in pulmonary vessels was demonstrated in patients with COPD or secondary pulmonary hypertension. In fact, platelet activation occurs in the systemic circulation of patients with COPD as a result of hypoxia, acidosis and hyperviscosity (20, 26). All of these symptoms are characteristic findings of chronic obstructive pulmonary disease. Also, platelet life-span was demonstrated to be shortened in COPD (21). It is suggested that the increased platelet activation seen in patients with pulmonary hypertension or COPD is due to a modification of anti-thrombotic properties of the endotelium and a change in vessel wall-platelet interactions, which are caused by increased shear-stress in the pulmonary vascular bed (20).

Our experiments were done in acute hypoxic conditions. It may be seen from the blood gas analysis that blood pH is within normal limits and acidosis is not present. In this condition, pulmonary hypertension observed during hypoxia is thought to be caused by serotonin, not released as a result of platelet activation, but released from NEB.

We did not observe a significant increase in pulmonary arterial pressure or serotonin levels during histotoxic hypoxia due to KCN injection. This shows again that NEB are not stimulated by histotoxic hypoxia (6, 7, 16). As NEB are not stimulated, no change can be observed in PAP and serotonin levels. On the breathing of the hypoxic gas mixture of the peripheral chemoreceptor-denervated dogs, BP was found significantly diminished. As is well known hypoxic hypoxia as well as histotoxic hypoxia increases the blood pressure by stimulating the peripheral chemoreceptors. After peripheral chemodenervation, hypoxic hypoxia causes a decrease in blood pressure by local vasodilator effect of decreased PO_2 in the tissues (10). Therefore the decrease in BP of the chemodenervated animals by hypoxic hypoxia can be attributed to this effect. In the absence of peripheral chemoreceptors KCN is not expected to cause a change in blood pressure. On the other hand, acute hypoxia does not possibly cause platelet activation to increase of BP.

In conclusion, our findings of the abolition of response of PAP to acute hypoxia after methysergide injection in peripheral chemoreceptor-denervated animals suggest that serotonin may be released from the NEB to contribute to the increase in PAP on the breathing of hypoxic gas mixture.

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