ORIGINAL ARTICLE

Chemoreceptor control of gas homeostasis in patients with obstructive sleep apnea

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Abstract

OBJECTIVE: The study was aimed at the determination of brain chemoreceptors sensitivity to hypercapnic stimulus in 15 patients admitted to Republican Scientific and Practical Centre of Otorhinolaryngology (Ministry of Health, Republic of Belarus) for examination and treatment of obstructive sleep apnea syndrome.

METHODS: Patients were divided into two groups according to the duration of the disease, body mass index and peculiarities of lung ventilation increase in short (3–4 minutes) inhalation of hypercapnic-hyperoxic gas mixture (rebreathing principle). The data obtained was compared with the results of 13 healthy volunteers. The role of central chemoreceptors in the initiation of the respiratory cycle under hypercapnia was demonstrated in acute experiments on 14 anesthetized Wistar rats, which had irreversible breathing arrest after 100 μl of 1% lidocaine hydrochloride application on ventral surface of medulla oblongata using intrathecally administered catheter.

RESULTS: The well-marked attenuation of growth of lung ventilation was stated in patients of both groups with obstructive sleep apnea under conditions of carbon dioxide increase in the expired air compared to healthy volunteers. This indicates the decrease in brain chemoreceptors sensitivity to carbon dioxide excess in the body.

CONCLUSIONS: Pathogenetic mechanisms of respiratory arrest during sleep in patients with obstructive sleep apnea in attenuation of brain chemoreceptors reactivity to hypercapnic stimulus are discussed.

Introduction

The problem of obstructive sleep apnea radical therapy is still a long way off from being resolved due to polyetiology of fatal phenomenon and the lack of data on fundamental mechanisms of irreversible breathing arrest development during sleep. A sleeping man is usually in a relaxed horizontal position, which facilitates the formation of airway obstruction by tongue, soft palate, excessive accumulation of saliva. The conditions for additive obturation of trachea and bronchi by relaxed airway muscles are formed due to wave-like changes of brain stem reticular formation tone during sleep (Abboud & Kumar 2014). Such processes are accompanied with disturbances in ventilation/perfusion relations in the lungs and the development

of hypoxia and hypercapnia as a consequence of this. The pathological shift of respiratory homeostasis (O_2 decrease, CO_2 and hydrogen ions increase) under normal conditions naturally leads to the activation of defense reflexes aimed at respiratory homeostasis recovery due to lung ventilation increase (Dempsey *et al* 2012; Sowho *et al* 2014). Dyspnea development is one of the signs of such reaction. The initiation of defense mechanisms is depressed or realized in perverted way in patients with obstructive sleep apnea syndrome. We will concretize the above. Air circulation disturbances due to airways obturation, the decrease in O_2 pressure in tissues and CO_2 level increase are unable to activate the mechanisms of vital functions control. These processes are turned on with long latent period in successful to patient's life situations and are accompanied with forced inspiration, distinctive sounds of snoring and short-term awakening of exhausted and feared (a kind of stress) patient due to reticular formation hyperarousal. By the way, snoring is associated by specialists not only with obstructive sleep apnea syndrome. A wide range of other situations foreign to the problem at issue may be the reason of snoring. Thus, namely in obstructive apnea during sleep (day or night) the signs of disturbances in sensitivity of central and peripheral chemoreceptors to respiratory homeostasis shifts are revealed.

It is worthwhile providing information on the hypothesis for central and peripheral chemoreceptors involvement in the development of obstructive sleep apnea syndrome onsets, when the desynchronization in their activity leads to fatal disruption of gas homeostasis (Dempsey *et al* 2012; Fiamma *et al* 2013). The authors of this hypothesis (Dempsey *et al* 2012) pay attention to the functional state of carotid chemoreceptors which predominantly respond to oxygen deficiency in the internal milieu (Fiamma *et al* 2013). The group of authors (Xie *et al* 2011; Fung 2014) tested the reactivity of respiratory centre in patients with obstructive sleep apnea syndrome and ascertained the fact of desensitization of both central and peripheral chemoreceptors. This, along with sympathetic tone increase can be treated as pathogenetic component of breathing arrests during sleep (Abboud & Kumar 2014). By the way, the pathological mechanism of apnea development in children is interpreted in a similar aspect (Goridis & Brunet 2010). Scientists pay attention on negative phases of long-term use of means allowing air inhalation under constantly increased pressure, as unfortunately it is accompanied with hypocapnia development (Haouzi & Bell 2009; Plataki *et al* 2013). Carbon dioxide is the natural activator of inspiratory activity central mechanisms generation (Guyenet *et al* 2010; Guyenet & Abbott 2013), and the decrease in its pressure in tissues is accompanied with attenuation of central chemoreceptors functional state and, as a consequence, with increase in the danger of breathing arrests of central origin development.

By the way, the events occurring in the internal milieu of professional deep water divers were compared to those in patients with obstructive sleep apnea syndrome (Ivancev *et al* 2007). The last ones demonstrated serious violations of respiratory homeostasis and pathological changes of blood vessels and sympathetic tone whereas divers had none of a kind, although the duration and consistency of breath holding are comparable in both groups.

Thus, chemoreceptor contour of vital functions control is the most important element in breathing activation and the modulator of sympathetic nervous system activation (Dempsey *et al* 2010). Main peripheral chemoreceptors are located in the area of carotid arteries bifurcation and respond mainly to hypoxemia. Central chemoreceptors are located in the area of brain stem and are included into breathing and blood circulation control in hypercapnia. The activation of chemoreceptor contour by both hyperoxic and hypercapnic stimuli simultaneously or by one of them is accompanied with hyperventilation and sympathetic nervous system activation (Abboud & Kumar 2014). Inhibitory vagal effect from the receptors of airways to respiratory centre neurons is cleared during apnea (the peak at a height of inspiration), and potentiation of sympathetic response to hyperoxic and hypercapnic stimuli takes place. Besides, the increase in sympathetic and ventilatory response to hypoxia is described in patients with hypertension (Iturriaga *et al* 2009). The increase in peripheral chemoreceptors sensitivity was noted in patients with obstructive sleep apnea and fatty people (Dempsey *et al* 2010). The role of central chemoreceptors in these processes is still being discussed. Hypersensibility to the lack of oxygen carry important consequences in patients with obstructive sleep apnea who suffer repeated stress in severe hypoxemia. Inhalation of 100% oxygen during sleep is accompanied with heart rate, blood pressure and sympathetic tone decrease in these patients. The reactivity of central chemoreceptors in patients with cardiac failure in hypercapnia changes leading to central apnea development during sleep (Dempsey *et al* 2010).

The lack of consensus on pathological mechanisms of obstructive sleep apnea development determined the aim of the study formation – to assess the reactivity of central chemoreceptors to hypercapnic stimulus in patients during functional blockade of vascular chemoreceptors and experimental apnea modeling by intrathecal application of anesthetic agent on ventral surface of medulla oblongata.

Material and Methods

The informed consent that can be used in scientific purposes was obtained from 15 patients and 13 healthy volunteers (male, aged from 40 to 60). The "rebreathing" test (Lipp *et al* 2010) was performed. The physician preliminarily instructed the volunteers on the peculiarities of the method. The data and time of study begin-

ning were systematically fixed in the protocol, as well as room temperature, barometric pressure level, humidity, name and surname of the patient, date of birth, sex, body mass and diagnosis. The name of the file for electronic fixation was also registered, as well as name and family of the physician.

All patients were placed in a comfortable sitting position. The intensity of tidal volume (ml kg^{-1}) and minute volume ventilation (L min^{-1}) growth were assessed every 2–4 minutes during the increase in carbon dioxide concentration (mm Hg) in inhaled air in "rebreathing" test, when the patient makes in- and exhalation from the closed place filled with oxygen. The dependence between minute volume ventilation and CO_2 level was approximated for every patient by linear function (y=k×x+b) to determine the intensity of ventilation growth in CO_2 increase (L min^{-1} $(mm\,Hg)^{-1}$), that was expressed with "k" coefficient. The mean, S.E.M. and standard deviation were calculated for every group. The statistical significance was evaluated by Mann-Whitney U-test.

The analysis of peculiarities of diaphragm electrical activity after subdural application of lidocaine hydrochloride (100 µl, 1%) and/or carbogen gas mixture (5% CO_2 and 95% O_2) inhalation was made in acute experiments on white male Wistar rats weighing 230–270 g (n=14). The Douglas bag (10 liters) filled with carbogen and connected to medical ventilator for small laboratory animals was used for creating hyperoxic-hypercapnic conditions. According to the protocol the exposition of gas mixture was from 1 to 5 minutes with subsequent ventilation of open air. Rats were intraperitoneally anesthetized with the mixture of urethane (500 mg kg^{-1}) and Nembutal (30 mg kg^{-1}), put into thermostatically controlled chamber and fixed after anesthesia at a heated operation pad (Braintree Scientific, Braintree, MA, USA). Tracheotomy was performed to connect medical ventilator. The electrical activity of diaphragm muscles was registered using bipolar silver-chloride electrodes. A short (about 1 cm) incision of abdominal wall was performed at the median line down from *processus xyphoideus* to access the diaphragm. Then the medisection of skin and soft tissues was performed at the anterior surface of the neck and silicone catheter (the outer diameter was 1.2 mm) was subdurally introduced in rostral direction 5 mm ahead through the formed aperture in *dura mater* of ventral region of atlanto-occipital joint. The termination of the catheter was oriented at the level of hypoglossal nerve roots output. Intrathecal bolus injection of lidocaine hydrochloride (100 µl, 1%, Sigma, St Louis, MO, USA) was made according to previously tried and tested technique (Semenik *et al* 2014). Deep body temperature was constantly maintained at the level of 37.5° C using thermocouple. The standard analysis of diaphragm electromyogram (Semenik *et al* 2014) was made using computerized electrophysiological device (WPI, FL, USA).

Results are expressed as mean ± S.E.M. Differences between means were evaluated by one-way analysis of variance (ANOVA) or Student's test for unpaired observations.

Results

The standard methodological technique of spirometabolographic analysis was used in patients' examining. The analysis was performed to assess the intensity of metabolism using indirect technique (by the ratio between eliminated carbon dioxide and consumed oxygen). The adaptation of this technique to the conditions of planned observations allowed realizing the protocol of registration. Realization of "rebreathing" technique is that as follows. Every trial subject began to inhale 100% oxygen from 10 liters Douglas bag. Increased oxygen concentration levels hypoxia development and provides functional blockade of peripheral (vascular) chemoreceptors which response to O_2 drop in the organism and are inhibited in hyperoxia (Sinski *et al* 2014). In- and exhalation was performed through the valve system directly to Douglas bag. As the nose clip prevented the initiation of nasal breathing, the whole cycle of external breathing was performed through the airways from the oral cavity to alveoli and back (closed contour). The concentration of oxygen in the Douglas bag decreases from breath to breath (but not lower than 180 mm Hg (Oxycount mini, Weinmann, Germany) being the guarantee of vascular chemoreceptors functional blockade) and carbon dioxide is accumulated (its level is determined by OLG-2800K capnograph (Nihon Kohden, Japan).

It makes sense to provide examples of examining patients and volunteers in order to illustrate the described technique. Namely the comparison of central chemoreceptors sensitivity to carbon dioxide (which is accumulated in the "rebreathing" test) was the key aspect of the study and subsequent multiple data analysis. Figure 1 shows the dependence of minute volume

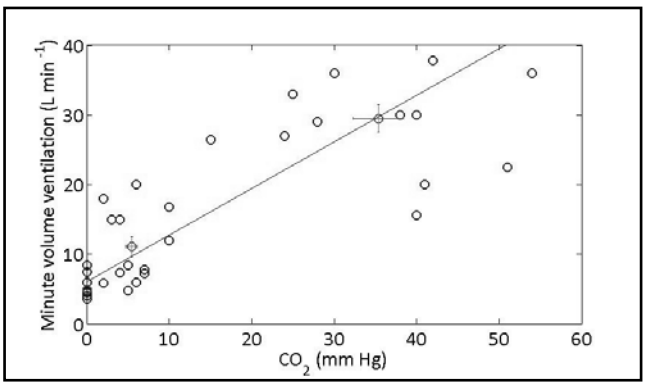

Fig. 1. The intensity of minute volume ventilation growth (L min^{-1}) during carbon dioxide increase (mm Hg) in expired air in healthy volunteers. The data are expressed as mean and standard deviation.

ventilation of healthy male volunteers on the level of carbon dioxide in inhaled air.

Figure 2 shows the dependence of minute volume ventilation (L min^{-1}) of patients with moderate obstructive sleep apnea on the level of carbon dioxide in the Douglas bag.

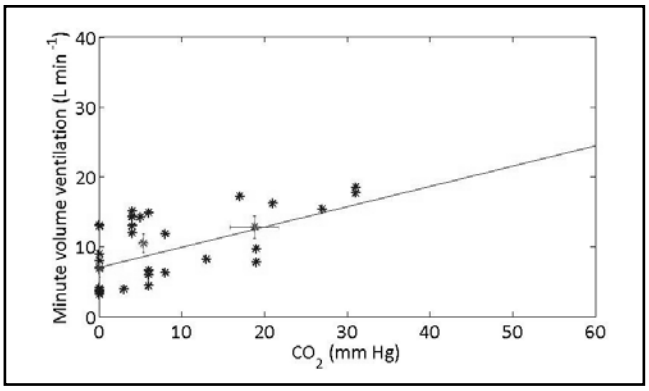

Fig. 2. The intensity of minute volume ventilation growth (L min^{-1}) during carbon dioxide increase (mm Hg) in expired air in patients with moderate obstructive sleep apnea. The data are expressed as mean and standard deviation.

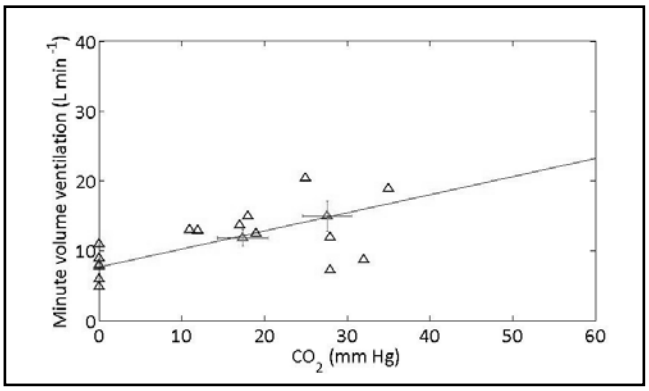

Fig. 3. The intensity of minute volume ventilation growth (L min^{-1}) during carbon dioxide increase (mm Hg) in expired air in patients with severe obstructive sleep apnea. The data are expressed as mean and standard deviation.

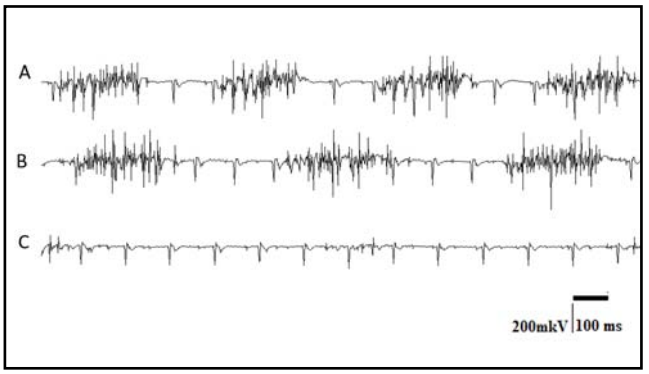

Fig. 4. Electrical activity of rat diaphragm before (**A**) and after 20 (**B**) and 60 (**C**) seconds after intrathecal application of lidocaine hydrochloride on ventral surface of medulla oblongata (100 µl, 1%).

The comparison of the slopes of the curves (Figure 1 and Figure 2) showed the decrease in minute volume ventilation growth in patients with moderate obstructive sleep apnea vs. healthy volunteers under the increase of CO_2 concentration in the inspired air (Figure 1 and Figure 2). When comparing the curves on Figures 1 and 2 conspicuous is the fact of rapid drop of minute volume ventilation growth (L min^{-1}) in patients with the signs of moderate obstructive sleep apnea. Such state is called hypopnea. The examination was performed in awaken people. Hypopnea frequently transforms into apnea of different duration during sleep. How does it happen? Breathing arrest is accompanied with the decrease of oxygen and the increase of CO_2 concentrations. Patients with obstructive apnea need more time to accumulate CO_2 to threshold compared to healthy volunteers, because of leveled ventilation intensity. Physicians know that sometimes such longstanding areactivity of respiratory centre accompanied with apnea may become fatal leading to death of the patient.

Figure 3 shows the dependence of minute volume ventilation (L min^{-1}) of patients with severe obstructive sleep apnea on the level of carbon dioxide in the Douglas bag. Thus, the comparison of data on Figures 1–3 shows the rapid decrease in medullary chemoreceptors sensitivity to hypercapnic stimulus in patients with obstructive sleep apnea. The experimental inhibition of central chemoreceptors activity was performed using the application of 1% lidocaine hydrochloride in order to confirm the hypothesis of central chemoreceptive mechanisms in apnea development.

Figure 4 shows the inhibition of diaphragm (main respiratory muscle) electrical activity in several seconds after intrathecal application of lidocaine hydrochloride (100 µl, 1%). Therefore, there is violation of conditions of respiratory rhythm generation after anesthetic agent application on the area of medullary chemoreceptors and subsequent breathing arrest in acute experimental conditions. Hypoxia and hypercapnia in anesthesia are unable to cause apnea stop in experimental animal. Carbogen (5% CO_2 and 95% O_2) inhalation was performed in anesthetized rats after apnea development and medical ventilator connection in order to confirm this hypothesis, additionally stimulate central chemoreceptors and initiate breathing. But there was no respiratory rhythm recovery after lidocaine hydrochloride application on ventral surface of medulla oblongata (the area of medullary chemoreceptors location). Besides, when comparing Figures 2 and 3 with Figure 1 the similar conclusion was made: the increase in lung ventilation in patients with obstructive apnea is attenuated under conditions of increasing hypercapnia during wakefulness.

Discussion

Obstructive sleep apnea syndrome is developed in examined patients only during sleep. Only attenuation of respiratory centre reactivity to hypercapnic stimulus

was stated in patients using "rebreathing" technique during wakefulness. It must be emphasized that such phenomenon develops namely in the state of wakefulness in the absence of apnea onsets. Literature data indicate that the change in central nervous system functional state during sleep makes fatal allowances to revealed patterns (Dempsey *et al* 2012). Therefore, the dysfunction of central mechanism of respiratory rhythm generation in patients with obstructive sleep apnea can be revealed even in the conditions of wakefulness during the analysis of lung ventilation at different levels of carbon dioxide in inhaled air (Onimaru & Homma 2003; Wittmeier *et al* 2008). It is worthwhile clarifying the character of development, frequency and duration of sleep apnea in patients in order to determine the tactics of individual therapy to prevent fatal conditions during sleep (Sowho *et al* 2014).

By this means, the type of dependence of lung ventilation growth from carbon dioxide concentration in inhaled air in healthy volunteers and patients with obstructive sleep apnea using multiple factor analysis of lung ventilation data, body mass, anamnesis and gas homeostasis parameters in "rebreathing" test. The data obtained correlate with the results of experimental and clinical studies of other scientists (Abboud & Kumar 2014) as well as conclusions of specialists in the field mechanisms of central and peripheral apnea development (Dempsey *et al* 2012; Fiamma *et al* 2013; Plataki *et al* 2013). All the examined patents with obstructive sleep apnea of different severity showed the attenuation of central chemoreceptors sensitivity to hypercapnic stimulus which is the evidence of the violation of central mechanisms of breathing regulation. Therefore, the general opinion on the necessity of surgical manipulations for peripheral mechanisms of obstructive sleep apnea correction proved to be shaken. The stated facts of dysregulation of central mechanisms of respiratory rhythm generation fairly in all patients with certain diagnosis of obstructive sleep apnea allow analyzing the pathological mechanisms of obstructive events formation and progression in airways from different positions. First of all, verification of central chemoreceptors dysfunction to hypercapnic stimulus in patients with obstructive sleep apnea is the reason for CPAP or BiPAP therapy prescription during natural sleep after additional monitoring of lung ventilation parameters in order to prevent fatal situations in the development of continuous apnea. Such tactics may sometimes become the only therapeutic outcome for the patient in severe functional state having a great number of comorbidities in order to prevent irreversible breathing arrest during sleep when there is no need of surgery.

An irreversible breathing arrest was demonstrated in acute experiments on anesthetized rats after the blockade of chemoreceptors on ventral surface of medulla oblongata with anesthetic agent (1% lidocaine hydrochloride). The experiment (Semenik *et al* 2014) is an additional argument for the key significance of central chemoreceptors in the activation of neurons in multicausal situations (e.g. in the violation of respiratory rhythm control in patients with obstructive sleep apnea syndrome) when the apnea develops and breathing does not resume despite the progressive CO_2 increase and O_2 decrease in the organism.

The attenuation of central chemoreceptors sensitivity to hypercapnic stimulus under conditions of changes of brain stem reticular formation functional state during sleep is accompanied with lowering of sensory input from brain chemoreceptors to respiratory centre neurons (Guyenet *et al* 2010; Guyenet & Abbott 2013). The initiation of respiratory activity in respiratory centre under these conditions depends on the degree of chemoreceptors sensitivity violation to carbon dioxide shifts in the organism. The more the sensitivity is weakened, the longer there will be the latent period of breathing initiation during apnea development in patients with obstructive events in the airways. Therefore, the determination of brain chemoreceptors sensitivity to hypercapnic stimulus allows assessing the degree of central mechanisms involvement into apnea development in patients with obstructive sleep apnea syndrome. The objective conditions are formed on the base of these data for substantiated use of CPAP or BiPAP therapy in those examined patients who suffer from the violation of brain chemoreceptors functioning, which is naturally accompanied with the threat of continued and fatal sleep apnea development.

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