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PARTICIPATION OF LEPTIN IN RESPIRATORY REGULATION AT THE LEVEL OF THE PRE-BÖTZINGER COMPLEX

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Annotation. Leptin, produced by adipocytes, has a significant dose-dependent stimulating effect on respiration when injected into the nucleus of the solitary tract. In this study we investigate respiratory reactions to microinjections (200 nl) of 0,1 nM, 10 nM, and 1 μM leptin into the Pre-Bötzinger complex, which is involved in the generation of the respiratory rhythm. In the course of our work, we found that microinjections of leptin (10 nM and 1 μM) into the pre-Bötzinger complex caused a dose-dependent increase in the minute volume of respiration primarily due to an increase in the respiratory rate without pronounced changes in the tidal volume or the maximum amplitude of the diaphragm or integrated EMG of the external intercostal muscles. The increase in respiratory rate was largely the result of a significant dose-dependent decrease in expiratory duration, while inspiratory duration did not change significantly.

Introduction. Discovery of the anorexigenic hormone called leptin (from the greek word "leptos" – delicate, beautiful, thin), which was revealed in 1994, became an important event in modern physiology.

Leptin regulates production and accumulation of fatty tissues in the organism, as well as sex-based features of the redistribution of fatty tissues [1]. Obesity could be explained by the disruption of its secretion in the organism. It was revealed in 1994 that secretion of this hormone is related to the OV-gene presence, which participates in the obesity's pathogenesis [2]. Leptin receptors on the brain's cell membranes were found later: in the hypothalamus (in the arcuate and ventromedial nuclei),the thalamus and the piriform cortex [3, 4].

We showed in our previous research that leptin microinjections directly into the "respiratory" area of the solitary tract's nucleus cause a significant dose-dependent respiratory stimulation, which is manifested through the increase of

the respiratory volume and the amount of comprehensive activity of inspiratory muscles without significant changes of the respiratory rate or the duration of respiratory phases [5, 6]. The stimulating respiratory effect of leptin on the tidal volume was achieved through the inhibition of the Hering-Breuer reflex, as well as the enhance of the respiratory reaction to CO_2 stimulation of central chemoreceptors located in the solitary tract's nucleus [6].

The pre-Bötzinger complex was described in 1991 by the international group of researchers as the area of the respiratory rhythm's generation [7]. It was established experimentally that structures generating the respiratory rhythm were found in the local area of the ventrolateral part of the medulla oblongata more caudal than the retrofacial nucleus [8, 9, 10, 11, 12]. The pre-Bötzinger complex in cats in vivo, which is located in the rostral part of n. ambiguus and the ventrolateral part of the reticular formation, that is more caudal than n. retrofacialis and more rostral than n. lateralis reticularis, was described in 1995 [13].

Therefore, we suggested that introduction of leptin to this structure can play a significant role in the respiratory rhythm modulation.

The purpose of our study was to examine respiratory reactions of leptin in case of microinjections into the pre-Bötzinger complex.

Methods and organization. The experiments were carried out in accordance with bioethical rules and standards of animal treatment and use in scientific purposes. They were conducted on 12 adult eats of both sexes with the body mass of 200-260 g. The rats were anaesthetized with urethane (1,5 g/kg abdominally, in a quantity of 3 ml/kg). Then the tracheostomy was performed. The tracheal cannula was inserted in the lower part of the trachea. The animal was fixated in the stereotaxic frame for small laboratory animals. Trepanning was performed in order to open the dorsal surface of the medulla oblongata.

We used glass micro droppers with an end diameter of 20-30 μm for leptin microinjections or the artificial cerebrospinal fluid (control microinjections) [14, 15]. The micro syringe MS-1 was used to make microinjections of leptin or the control fluid into the area of the pre-Bötzinger complex. Leptin was dissolved in the artificial cerebrospinal fluid until the 0,1 nM, 10 nM, and 1 μM concentration. The fluid was injected with a constant rate in the quantity of 200 nl.

Respiration parameters were evaluated according to the spirogram and the electromyogram (EMG) of the diaphragm and external intercostal muscles. The spirogram was registered using the miniature spirograph connected to the tracheal cannula. Then, a signal from the spirograph was registered and recorded on a computer. Using the spirogram, tidal volume (Vt, ml), inhale time (Ti, s) exhale time (Te, s) and total time of the respiratory cycle (Ttot, s) were recorded. Then,

respiratory rate (f, exhale/min) is calculated as 60 s/Ttot, the respiratory minute volume was calculated as the product of Vt and f.

The EMG recording was conducted using bipolar steel needle electrodes. The diaphragm EMG was registered through the cut in the frontal abdominal wall under the ribcage on the right side. The EMG of external intercostal muscles was registered in the sixth-eighth intercostal space. The electromyogram was registered using the electromyograph and recorded, integrated on a computer. The respiratory activity amplitude was used as a mean of electrical activity of inspiratory muscles. The spirogram and EMG were recorded continuously during the whole experiment, initial values of respiratory parameters (for 30 s in average before the microinjection) were compared with the new ones in the 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50 and 60 min after the microinjection.

At the end of every experiment the rat was sacrificed, the brainstem was removed and placed into a fixator (6% formaldehyde in the physiological liquid) for 2 weeks. After that, frozen sections were made (50 μm) to check stereotaxic located points of the microinjection according to the Paxinos & Watson atlas [16].

Statistical and graphical analysis of data was conducted using the software package SigmaPlot. Analysis of answers on leptin microinjections was carried out using the ANOVA test for repeated measurements. If there were statistically significant changes, following a posteriori comparisons with initial values (before leptin injections) using the Holm-Sidak test were conducted. The Kolmogorov-Smirnov test was used to define whether a selection belongs to the population with normal distribution. All values were shown as mean values ± the standard error of the mean (SEM). The statistical significant was noted if $P<0,05$.

Results and discussion. The leptin microinjection into the area of the pre-Bötzinger complex caused a dose-dependent stimulation of respiration. Injection of 0,1 nM leptin did not cause statistically significant changes in the pattern of respiration or in the EMG of inspiratory muscles. Since such leptin concentration did not cause respiratory reactions, we consider it subliminal. Control microinjections with the artificial cerebrospinal fluid into the area of the pre-Bötzinger complex also did not cause changes in respiratory parameters. Respiratory reactions of the external respiration and the EMG of inspiratory muscles to 10 nM leptin microinjections are presented on fig. 1.

The 10 nM leptin microinjection caused a moderate stimulation of respiration. This effect was shown through the progressing increase of ventilation ($P<0,01$: the ANOVA test), which reached its maximum approximately 15 minutes after the leptin injection (Fig. 3). Vi was increased from 64,3±2,8 to 73,1±2,9 ml/min ($P<0,01$: the Holm-Sidak test) on the effect's peak. The Vi change was caused by the f increase ($P<0,01$: the ANOVA test), since Vt did not change at all (Fig 2). The

respiratory rate was progressively increasing, and the maximum change in f happened 15 minutes after when f increased from 61,2±2,8 to 72,4±3,5 exhales/min (P<0,01: the Holm-Sidak test). The respiratory rate remained increased and significantly exceeded the initial value for a period of 30 minutes after the injection. Analysis of indicators of respiratory time showed a decrease of Ttot as a result of Te's shortening. The exhale time was gradually decreasing (P<0,05: the ANOVA test) due to the increase in f. When the effect peaked, Te decreased from 0,72±0,04 to 0,59±0,04 s (P<0,01: the Holm-Sidak test). There was no significant change in Ti after the 10 nM leptin injection. The maximum amplitude of the integrated diaphragm EMG and external intercostal muscles also remained unchanged after the 10 nM leptin effect.

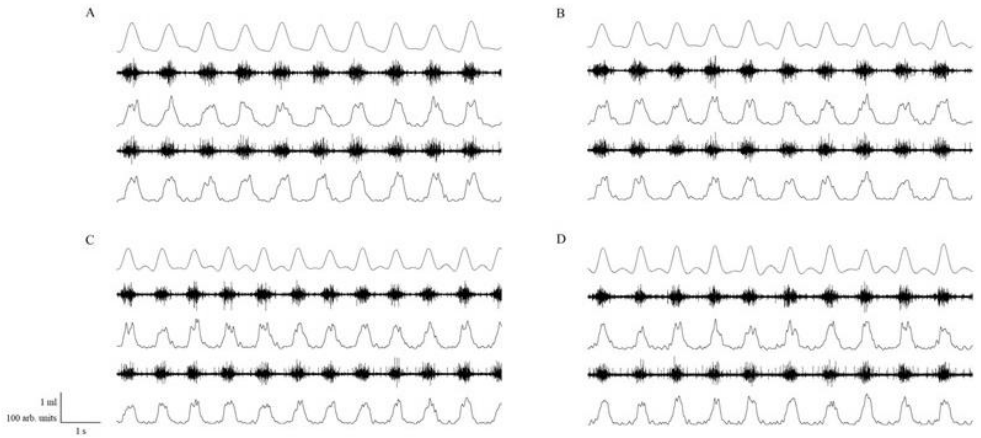

Fig. 1. Respiratory reactions to 10 nM leptin microinjections into the pre-Bötzinger complex

Note: A – initial state; B – 5 minutes; C – 20 minutes; D – 40 minutes after the leptin injection. Fragments of curves are presented from top to bottom: spirogram, diaphragm EMG, integrated diaphragm EMG, EMG of external intercostal muscles, EMG of integrated external intercostal muscles

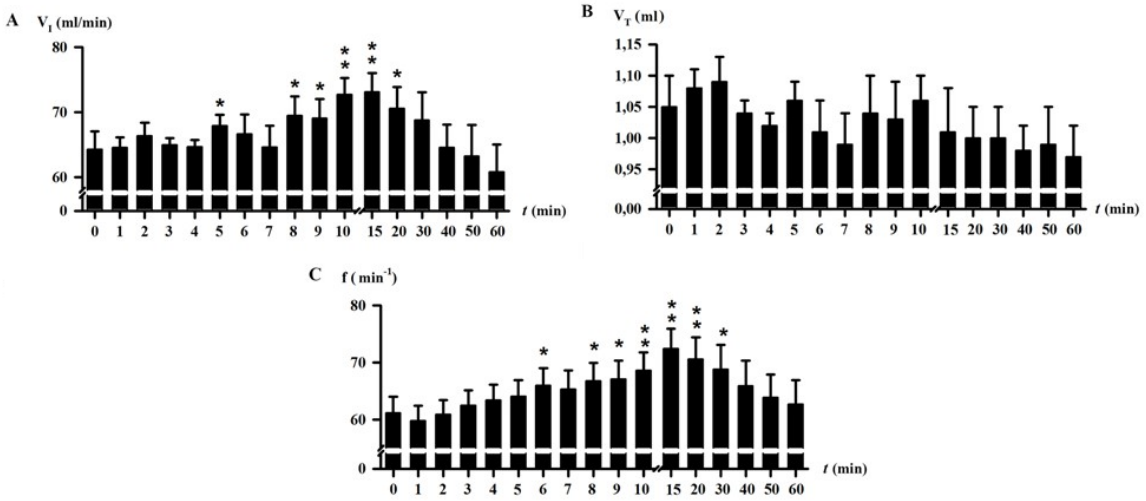

Fig. 2. Respiratory minute volume (A), tidal volume (B) and respiratory rate (C) in the initial state (0) and in various moments of time (1 to 60 minutes) after the 10 nM leptin injection into the pre-Bötzinger complex

Note: * – P<0,05, ** – P<0,01

Fig. 3 shows the change in the respiration pattern and in the EMG of the diaphragm and external intercostal muscles to 1 μM leptin microinjection into the pre-Bötzinger complex. The 1 μM leptin injection led to the significant stimulation of respiration, causing a gradual increase in the respiratory minute volume (P<0,001: the ANOVA test), which became maximal 20 minutes after the leptin injection (Fig. 4). The respiratory minute volume was increased from 77,2±3,2 to 95,5±4,9 ml/min (P<0,001, the Holm-Sidak test). The Vi increase was related to f's increase (P<0,001: the ANOVA test) from 64,4±2,7 to 82,3±4,6 inhales/min. The effect's beginning lasted 3 minutes, the maximal change in the respiratory rate happened approximately after 20 minutes, the full recovery was recorded within 50 minutes (Fig. 4). There were no obvious changes in Vt (Fig. 4) or in peak amplitude of the integrated EMG of the diaphragm or external intercostal muscles after the leptin microinjection on the 1 μM concentration. Increase in the respiration rate to a significant degree was a result of a significant shortening of Te. Ti did not change significantly. The exhale time was gradually decreasing (P<0,001: the ANOVA test), reaching its maximal value (approximately 20 minutes after) from 0,70±0,04 to 0,54±0,05 s (P<0,01: the Holm-Sidak test).

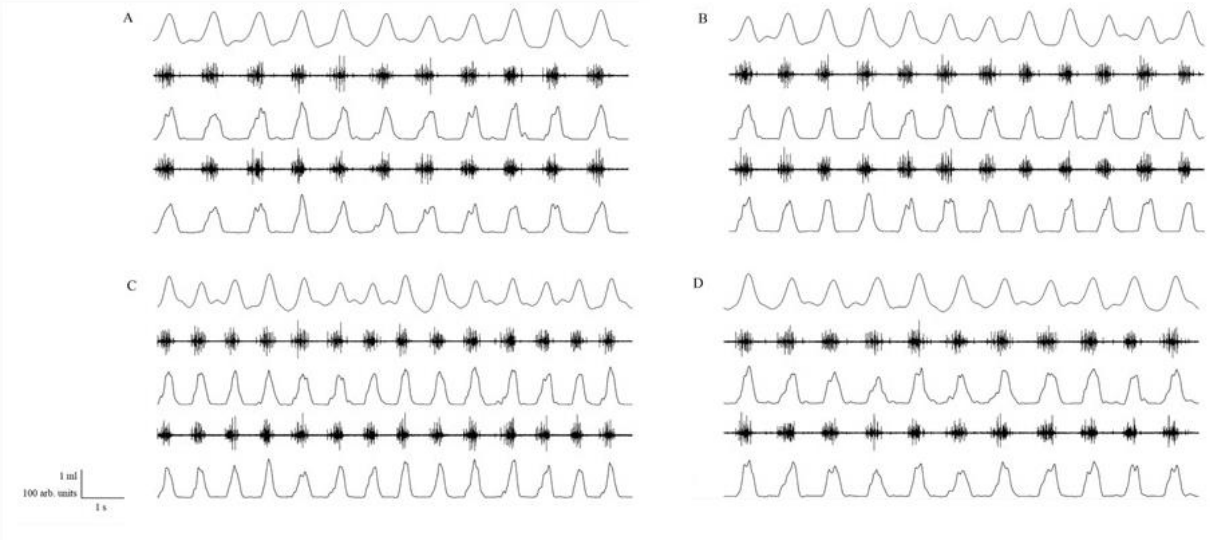

Fig. 3. Respiratory reactions to 1 μM leptin microinjections into the pre-Bötzinger complex

Note: A – initial state; B – 5 minutes; C – 20 minutes; D – 40 minutes after the leptin injection. Fragments of curves are presented from top to bottom: spirogram, diaphragm EMG, integrated diaphragm EMG, EMG of external intercostal muscles, EMG of integrated external intercostal muscles

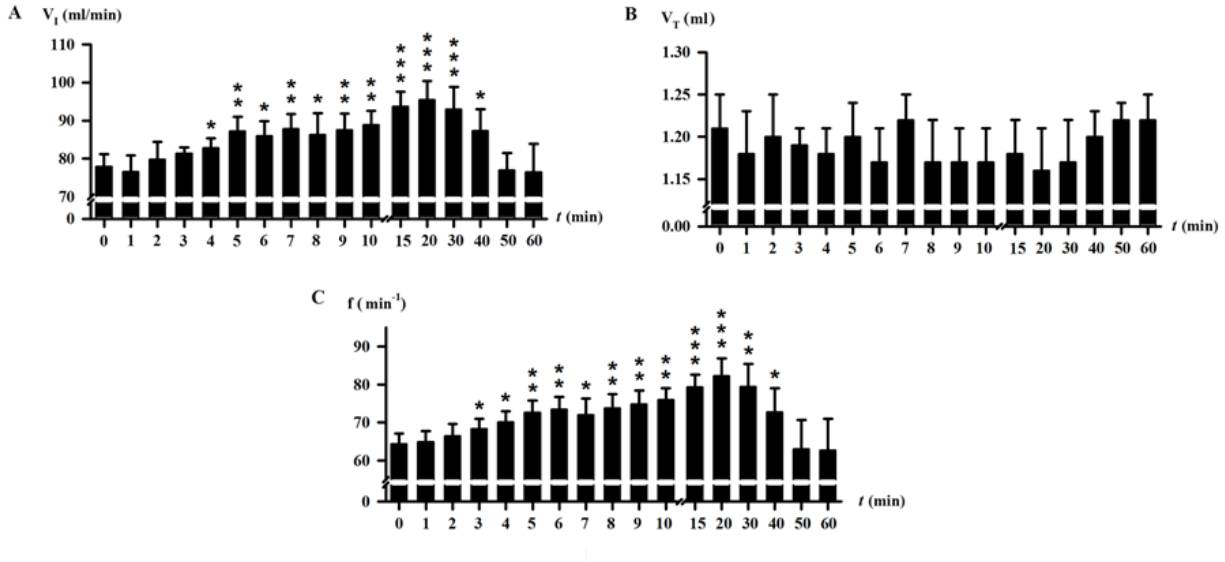

Fig. 4. Respiratory minute volume (A), tidal volume (B) and respiratory rate (C) on the initial level (0) and in various moments of time (1 to 60 minutes) after the 1 μM leptin injection into the pre-Bötzinger complex

Note: * P – <0,05, ** P – <0,01, *** – P<0,001

The results obtained confirm earlier described stimulating respiratory effects of leptin [5, 6, 17]. The data obtained demonstrates that leptin has a direct stimulating effect on respiration in case of microinjections into the pre-Bötzinger complex. After leptin microinjections, the respiratory minute volume was decreased, firstly as a result of a significant increase of the respiratory rate, not the tidal volume. However no changes in the peak amplitude of the integrated EMG of the diaphragm or external intercostal muscles were registered. A significant shortening of the exhale time was registered, while the inhale time did not change. The range of effective leptin concentrations in the injected fluid was rom 10 nM to 10 μM, 0,1 nM leptin concentration did not cause any respiratory reactions.

Conclusion. The presented study serves as an evidence that leptin can make a stimulating respiratory effect in case of microinjections into the pre-Bötzinger complex, playing an important role in the respiratory rhythm generation. With our previous studies and present literature data, these results suggest that leptin can participate in processes of the respiratory regulation alongside with metabolic processes to stimulate ventilation through the pre-Bötzinger complex.

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