International Journal of Sciences

Research Article Volume 5 – July 2016 (07)

Effect of Orexin-A on Glucose Sensitive Neurons in the hypothalamus of Rats

Mengling Zhang[1], Xiangrong Sun[1], Feifei Guo[1], Shengli Gao[1], Yanling Gong[2], Luo Xu[1]

[1]Department of Pathophysiology, Medical College of Qingdao University, Qingdao, Shandong, China

[2]Department of Pharmacy, College of Chemical Engineering, Qingdao University of Science and Technology, Qingdao, Shandong, China

Abstract: Orexin-A is an excitatory neuropeptide that mainly located in the lateral hypothalamus(LHA). Orexin-A can regulates multiple physiological activity that include arousal, wakefulness and appetite. We aim to discuss the effects of orexin-A on the glucose sensitive neurons in the hypothalamus, in order to make further investigation of potential mechanisms in the appetite system. Cerebral stereotaxic technique was used to inject orexin-A and glucose into nucleus accumbens of rats. Extracellular single unit discharges were used to observe the activity of glucose sensitive neurons in the nucleus accumbens of rats. Sixty-eight glucose sensitive neurons were recorded after microinjection of glucose into nucleus accumbens. Based on different sensitive reactivity, the discharge frequency of forty-two neurons were increased (defined as glucose excitatory neuron) and twenty neurons were decreased (defined as glucose inhibitory neuron). The discharge frequency of thirty neurons were increased in the glucose excitatory neurons after administration of orexin-A through four-barrel glass microelectrode. The discharge frequency of fifteen neurons were increased in the glucose inhibitory neurons after administration of orexin-A through four-barrel glass microelectrode. The effect of orexin-A on turning the discharge frequency of glucose sensitive neurons was abolished by orexin-A receptor antagonists SB-334867. The change of discharge frequency of glucose sensitive neurons did not have significant difference after injection of saline through three-barrel glass microelectrode. It is suggested that orexin-A can regulate glucose sensitive neurons in nucleus accumbens, which may be parts of mechanism of the regulation of food intake.

Keywords: orexin-A; feeding; glucose sensitive neuron

Introduction

The orexins system is made up of the two orexin-neuropeptides (orexin A and orexin B) and two G-protein-coupled receptors (the orexin-1 and the orexin-2 receptor) [1]. Orexin-A (OXA) and orexin-B (OXB) are both produced from same prepro-orexin in the lateral hypothalamus[2]. Orexin-A positive neurons mainly located in the lateral hypothalamus(LHA) and the perifornical area(PeF) [1]. But its nerve fibers have widespread project into brain regions, such as paraventricular nucleus(PVN), ventromedial nucleus(VMH), hippocampus, nucleus accumbens(NACC) [3]. Orexin-A is an excitatory neuropeptide that is participate in variety of physiological functions, including arousal, reward-addictive behaviors, appetite, neuroendocrine homeostasis, and balance between metabolism and energy expenditure [4,5]. Recent studies have shown that orexin-A can regulate activities of gastric distention-sensitive neurons and gastric motility [6]. Moreover,

Published at: http://www.ijsciences.com/pub/issue/2016-07/
DOI: 10.18483/ijSci.1108; Online ISSN: 2305-3925; Print ISSN: 2410-4477

Luo Xu (Correspondence)
xu.luo@163.com
+86 532 82991713

there is evidence that orexin-A in nucleus accumbens (NAcc) could be involved in feeding and drinking [7]. However, the mechanism that orexin-A can regulate energy balance has not clear enough, needing a further researched. It is reported that glucose sensitive neurons are capable of sensing the level of glucose intracellular and extracellular, and then feedback on the homeostasis balance system. Here, we aim to examine whether glucose sensitive neurons exists in NAcc and whether orexin-A administration into NAcc affects glucose sensitive neurons. We hope that these studies can perfect the regulatory mechanism of orexins system on emergy balance.

Methods

Animals

Adult male Wistar rats (Qingdao Institute for Drug Control, Shandong, China) were used, weighting 250-300g. All rats were housed in a controlled room with temperature (25±3℃) and the 12-12 light-dark cycle. All rats intake food and water freely during the experiment. Before the experiment, rats have given adaptation at least 7 days. The study was approved by Animal Care and Use Committee at Qingdao University, and all procedures were performed in accordance with institutional guidelines.

Group of experiments

In our study, fifty rats were divided into two groups randomly: the sham group (n=20) and the experiment group (n=30). Rats of the sham group were given saline to glucose sensitive neurons. The experiment group was given 0.5 μg/0.5 μL orexin-A or 6.0 μg/0.5 μL SB-334867 to glucose sensitive neurons.

Electrophysiological experiment

After fasting for 18 h, 10% chloralic hydras was used to anaesthetize rats, and then placed rats in a stereotaxic apparatus (Narashige SN-3, Tokyo, Japan). Next, we had removed their head fur, exposed their skull, adjusted bregma and posterior fontanelle to the same horizontal, and drilled skull to expose brain parenchyma. The open part of the brain was covered with warm agar (3% in saline) to improve stability for neuronal recording. Multi-barrel glass microelectrode（total tip diameter 3–10 μm, resistance 5–20 MΩ） were inserted into the area of NAcc (bregma: P: 2.0 – 2.3 mm, L (R): 1.4 – 1.6 mm, H: 7.2 – 7.6 mm) [8] for extracellular single unit recordings and micropressure injection. The recording barrel of the electrode was filled with 0.5 M sodium acetate and 2% pontamine sky blue.

A four-barrel glass microelectrode was inserted in experiment group. Besides the recording electrode, the other three barrels connected with a three-channel pressure injector (PM2000B, Micro Data Instrument, Inc., USA), were filled with a 15 nM solution of orexin-A, a 25 nM solution of SB-334867, and glucose solution, respectively. A three-barrel glass microelectrode was inserted in sham group. Besides the recording electrode, the other two barrels were respective filled with saline and glucose solution. Drugs were ejected onto the surface of firing cells with short pulse gas pressure (1500 ms, 5.0–15.0 psi) [9] .The intra-barrel drug concentrations were based on their efficacy in reliably altering cell firing. Volumes <1 nL of orexin-A or other drugs were applied to the firing cells during extracellular recordings.

After the microelectrode was advanced into NAcc, the extracellular action potentials of single neurons were recorded, amplified using a high input impedance amplifier (MEZ8201; Nihon Kohden, Tokyo,Japan), and displayed on an oscilloscope (VC-11, Nihon Kohden). 0.5mol/L glucose was injected into neurons through the multi-barrel glass microelectrode, and then observed the change of the firing rate of neurons. A neuron was identified as a glucose-sensitive neuron if its mean firing frequency changed via glucose by at least 20% from the mean basal firing level. The glucose-sensitive neuron were further classified into glucose excitatory neuron and glucose inhibitory neuron according to whether the spontaneous discharge increased or decreased by the administration of glucose. In experiment group, orexin-A was administrated by the four-barrel glass microelectrode until the firing rate

returned to normal and stabilized at least 120s, then observed 3-4 minutes. Then, SB-334867 was administrated in the same way and also observed 3-4 minutes. In sham group, saline was injected through the three-barrel glass microelectrode.

Histological verification

At the end of each experiment, a direct current (10 μA, 20 min) was passed through the electrode to form an iron deposit of pontamine sky blue into the recording site to verify the position of the recording electrode. After perfusion and fixation of the brain, coronal 50μm frozen coronal sections were cut through the regions of the NAcc to observe the position of injection via microscope. Those with incorrect locations were excluded from analyses.

Statistics

All data were presented as mean ± SD. SPSS 17.0 statistics software was used to process the data. The difference in the firing rate of the same unit neuron before and after treatment were compared by paired Student's t-test.

Results

Effects of glucose on discharges of neurons in the NAcc of rats

Spontaneous unit discharges of 68 neurons in the NAcc were recorded. After microinjection of glucose, the discharge frequency of 42 neurons had increased with statistical significance (7.35 ± 1.42 Hz vs 14.25 ± 2.21 Hz, $p < 0.05$). They were identified as glucose excitatory neuron. The discharge frequency of 20 neurons had decreased with statistical significance (7.92 ± 1.71 Hz vs 3.04 ± 1.01 Hz, $p < 0.05$) after microinjection of glucose which were identified as glucose inhibitory neuron.

Effects of orexin-A on discharges of glucose sensitive neurons in the NAcc of rats

Out of the 42 glucose excitatory neurons, after orexin-A was administrated to the NAcc, 30 neurons were excited with a increased firing rates from 7.51 to 13.26. And 9 neurons were inhibited with a decreased firing rates from 10.01 ± 1.61 Hz to 5.13 ± 1.31 Hz, the difference had statistical significance ($p < 0.05$), and 3 neurons failed to respond to orexin-A clearly (Fig.1A). Out of the 20 glucose inhibitory neurons, orexin-A was administrated to 17 neurons through a glass microelectrode, 15 neurons excited by orexin-A had firing rates which increased from 8.19 ±1.27 Hz to 15.24 ± 1.10 Hz, the difference had statistical significance ($p < 0.05$), and 1 neurons failed to respond to orexin-A clearly (Fig.1B).

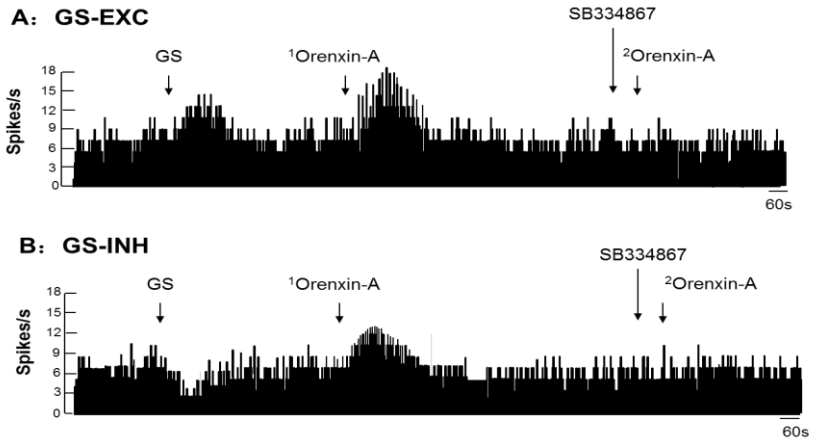

Fig.1 Effect of orexin-A on firing frequency of glucose sensitive neurons in NAcc

Application of orexin-A caused a remarkable increase in neuronal firing frequency of GS-EXC neurons (A) and GS-INH neurons (B). After SB-334867 pretreatment the responses induced by orexin-A were completely diminished.

Upon microinjection of the mixture of orexin-A and SB-334867 on glucose sensitive neurons in the NAcc, the discharge frequency had almost no change. After microinjection of saline into glucose sensitive neurons in NAcc through a three-barrel glass microelectrode, there were no significant changes in the discharge frequency in sham group.

Discussion

The regulation of ingestion and energy is always in a dynamic balance under normal circumstances and thereby maintain the energy homeostasis. Numerous peptides are involved in these metabolic processes, such as orexin which can promote feed intake, NPY, leptin, melanin concentrating hormone and nesfatin-1[10]. Fibers of orexin neurons project throughout the brain and orexins receptor widely distributed in the brain, which includes NAcc[3]. Moreover, NAcc is regarded as "pleasure centre" which become more active in response to a series of stimulation, such as food, sex and drug. Glucose sensitive neurons can apperceive the change of the level of extracellular glucose which is bound up with feeding, and then triggered multiple somatic and non-somatic responses, like initiate or terminate feeding behavior, in order to maintain the body's energy balance[11].

We observed that glucose sensitive neurons were existed in NAcc, and administration of orexin-A into the NAcc through grass microelectrode can increased the firing activity of glucose inhibitory neurons and glucose excitatory neurons in this experiment. These effects are eliminated with orexin-A receptor antagonist SB-334867. It suggests that orexin-A can modulate the activities of glucose sensitive neurons, maybe influence satiation signals furtherly.

Recent studies have shown that some neurons in the hypothalamus and dorsal vagal complex are sensitive to the changes of extracellular glucose concentration, and they can change their discharge frequency according to glucose as a signal[10]. Other studies have shown that glucose sensitive neurons in the hypothalamus and NTS play a role on the regulation of feeding[11,12]. Besides, there are some interactions between the central glucose sensitive neurons and other factors involved feeding, such as NPY and POMC[11,13,14]. Thus, we speculated that the central glucose sensitive neurons maybe one of the target neurons that orexin-A exert their effect, and this may be parts of mechanism of the regulation of food intake. The specific mechanism is not clear enough yet, and need to further research.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81470815, No. 81270460, No. 81300281 and No. 81500414); Qingdao Municipal Science and Technology Commission (14-2-3-3-nsh), Research Award Fund for Outstanding Middle-aged and Young Scientist of Shandong Province (No. BS2014YY009).

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