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ARTICLE

INCRETIN MAY BE INVOLVED IN THE PROCESS OF OPIOID-INDUCED HYPERALGESIA IN RATS

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ABSTRACT

In this paper, we investigated whether incretin is involved in the process of opioid-induced hyperalgesia (OIH) in rats. The OIH model was prepared by subcutaneous injection of fentanyl citrate in the rat neck. Then, isolating Root 's ganglion (dorsal root ganglion, DRG) cells acutely from the L4-L6 segments of the lumbar spine, we found that the GIP gene in the DRG of the OIH group was significantly up-regulated compared with the control group, while the GLP-1 gene had no significant change compared with the control group by real-time quantitative PCR. Indicating that the GIP gene in incretin may be involved in the peripheral sensitization process of peripheral rats.

KEY WORDS

Opioids; Hyperalgesia; Incretin; GIP; GLP-1

1. INTRODUCTION

Opioids are important drugs for clinical anesthesia and moderate to severe pain management. In 1880, Rossbach proposed that long-term use of opioids would have the exact opposite effect of analgesia[1]. Subsequent studies have shown that in addition to their powerful analgesic effects, opioids can also sensitize central and peripheral nociceptive pathways and increase the body's sensitivity to nociceptive pain, known as opioid-induced hyperalgesia(OIH)[2].

The dorsal root ganglion (DRG) is the distended nodule of the dorsal root of the spinal cord, and is the primary afferent element of pain conduction, with the functions of transmitting and regulating body sensation, reception and conduction of nociception[3, 4]. The current research on the involvement of DRG in pain mechanisms mainly focuses on neuronal ion channels, glial cells and inflammatory responses.

Glucagon-like peptide-1 (GLP-1) synthesized and secreted by ileal L cells and glucose-dependent insulin-stimulating polypeptide (GIP) synthesized and secreted by duodenal and jejunal K cells are collectively known as incretin. Incretin can stimulate the secretion of insulin, but also inhibit the secretion of glucagon after meals, enhance insulin sensitivity, delay gastric emptying, promote the proliferation of pancreatic β cells, and suppress appetite[5, 6]. In recent years, studies have found that incretin and its receptor system are involved in the formation of central nervous system plasticity and play an important role in the learning, memory and central sensitization of pain[7-10]. In the process of hyperalgesia, peripheral sensitization is also a very important part, and there are few reports on the involvement of incretin and its receptors in peripheral sensitization.

In this paper, we used real-time PCR to find that GIP in DGR cells of OIH rats was up-regulated compared with the control group, while GLP-1 had no significant change, suggesting that the GIP in rat DRG cells may be involved in large-scale OIH process in mice.

2. MATERIALS AND METHODS

2.1 Animals and treatment

Six three-week-old male Sprague Dawley (SD) Rats (60-80 g) were provided by the Laboratory Animal Center of Hubei Provincial Center for Disease Control and Prevention. They were housed in separated cages (20℃-22℃, fed ad libitum, and maintained on a 12-hour light/12-hour dark cycle). Rats were numbered according to random number table, ranked by the ascending order and randomized into two groups：Control and OIH. All procedures involving animals were performed with the approval of the Laboratory Animal and Biomedical Ethics Committee of South-Central University for Nationalities (number: 2019-SCUEC-AEC-022).

2.2 Preparation of the OIH rat model

As described previously[11], four times injections of fentanyl (60 μg/kg per injection) at 15 min intervals, resulting in a cumulative dose of 240 μg/kg were conducted to induce OIH in this study. The control animals received an equal volume (1.2 ml/kg) of physiological saline.

2.3 Behavior testing

Mechanical sensitivity was tested using the von Frey filaments (North Coast, USA) according to the Dixon method[12]. Before the test, rats were placed into separate Plexiglas containers over mesh platforms and allowed to adapt about 30 min to achieve immobility. Beginning with

0.4 g, fibers of sequentially increasing stiffness were perpendicularly applied to the mid-plantar surface according to the up-down paradigm. Positive response was defined as paw flinching shook, or licking its paw or brisk withdrawal. The interval between adjacent tests was more than 5 minutes. The 50% probability of paw withdrawal threshold (PWT) was calculated using the up and down paradigm.

2.4 Dorsal root ganglia isolation

DRG neurons from rat were dissociated as described previously[13]. In brief, the rat were killed by decapitation. Lumbar DRGs (L4–L6) were quickly excised and placed in ice-cold DMEM. Cut off the excess connective tissue around the DRG cells under a stereoscope, and place the cleaned DRG in another pre-cooled petri dish.

2.5 Real-time quantitative PCR

The total RNA of the DRG was extracted using TRIzol reagent (Invitrogen, USA) as described previously[14]. Reverse transcription was performed using GoldenstarTM RT6 cDNA Synthesis Kit (TsingKe, China). Quantitative polymerase chain reaction (qPCR) analysis was performed in a real-time detection system (Agilent Technologies Stratagene Mx3005P, USA) by TB Green™ Premix Ex Taq™ II (Takara, Japan). The following primers were used: Gip forward, 5'-GACTGGACTCTGACCTTAG-3'; Gip reverse, 5'-GGCTTTATTGGTTTGGTTC-3'; Glp-1 forward, 5'-TTACATCGTGGCTGGATT-3'; Glp-1 reverse, 5'-TCTTCAAGTGGTTCTGTCT-3'; Hprt forward, 5'-CCTTGACTATAATGAGCACTTC-3'; Hprt reverse, 5'-AACAGGACTCTTGTAGATTCA-3'. PCR amplification was performed at 95°C for 30 s followed by 45 cycles of cycling at 95°C for 5 s and 60°C for 30 s. Hprt was used as an internal control for normalization. The ratios of mRNA levels were calculated using the $-\Delta\Delta Ct$ method ($2^{-\Delta\Delta Ct}$).

2.6 Statistics

GraphPad Prism 8 was used for statistical analyses. All of the results are presented as mean ± SEM. The behavioral data were analyzed by two-way ANOVA followed by the Bonferroni test. The qPCR data were analyzed by unpaired t-test. For all experiments, $P < 0.05$ was considered to be significant ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

3. RESULTS

3.1 Fentanyl significantly decreased paw withdraw threshold in rats

The rats in the control group were injected with saline, and the rats in the OIH group were injected with fentanyl. The time points for measuring PWT were selected as the time points before (0 h) and after the modeling (6.5 h)[15]. The PWT changes are shown in Figure 1. The results showed that the PWT in the control group had no significant difference before and after modeling; while, the PWT in the OIH group was significantly reduced 6.5 hours after modeling, confirming that the OIH rat model was successfully constructed.

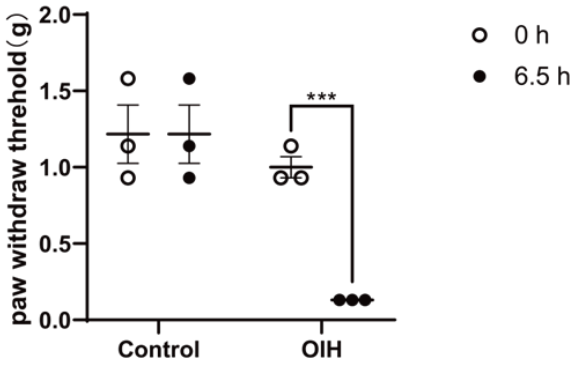

Figure 1: Changes of PWT before and after modeling

3.2 The mRNA expression of GLP-1 in DRG of OIH rats has no significant change

The RNA of DRG cells from L4-L6 lumbar vertebrae of rats in control group and OIH group were extracted for real-time quantitative PCR. The result is shown in Figure 2, there was no significant difference in the mRNA expression of GLP-1 in the DRG of OIH rats compared with the control group.

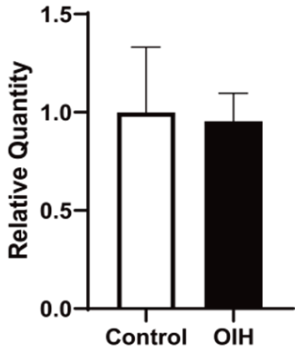

Figure 2: Changes of GLP-1 mRNA expression in DRG of OIH rats

3.3 GIP mRNA expression was significantly up-regulated in DRG of OIH rats

The result is shown in Figure 3, the mRNA expression level of GIP gene in the DRG of OIH rats was 4.11 times higher than that of the control group. P=0.013 after unpaired t-test, indicating that GIP was significantly up-regulated in the DRG cells of the OIH rats compared with the control group.

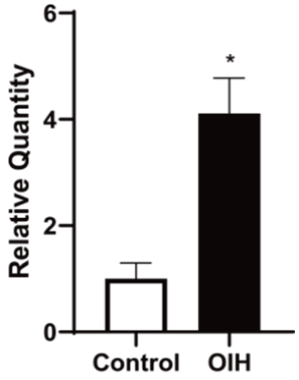

Figure 3: Changes of GIP mRNA expression in DRG of OIH rats

4. DISCUSSION

So far, there are some progress on the process of OIH, but its mechanism has not been fully elucidated. Current research suggests that the mechanism of OIH includes the following aspects: activation of central glutamatergic system, blockade of inhibitory neurotransmitter (such as GABA) receptor system, endogenous opioid peptides (such as dynorphin, *P* substances, cholecystokinin, etc.) activation, μ-opioid receptor (MOR) phosphorylation, facilitation of spinal descending pain regulation system, etc.[16-18]. OIH has no uniform clinical diagnostic criteria and is difficult to treat. The current recommended drug regimens for OIH are: NMDA receptor antagonists (such as ketamine, methadone), non-steroidal anti-inflammatory drugs (such as parecoxib), α2 adrenergic receptor agonists (such as clonidine) and Antiepileptic drugs (such as bapentin), etc.[19, 20]. In addition to drug treatment options, there are psychotherapy and nerve block therapy. But according to the mechanism of OIH, gene therapy options still need to be explored.

At present, the studies on GIP/GLP-1 gene and its receptor is mainly focused on the regulation of diabetes and obesity. A large number of studies have proved that GIP/GLP-1 gene and their receptors are involved in central nervous system plasticity and central pain sensitization[21, 22], while rarely reported in peripheral sensitization. GIP/GIP receptors and GLP-1/GLP-1 receptors have a cross mechanism in the regulation of pain. The activation of GIP signaling system contributes to pain sensitization, while GLP-1 signaling system may inhibit pain sensitization.

In this paper, the mRNA expression of GIP was found to be up-regulated

in DRG cells of OIH model rats, and the mRNA expression of GLP-1 gene did not change significantly, which provided a basis for the involvement of GIP and its receptors in peripheral pain sensitization. The cross-regulation mechanism of GIP and GLP-1 signaling may also be a new mechanism of peripheral pain sensitization, and the elucidation of its mechanism will contribute to the research and discovery of new analgesics.

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