Life Sciences 203 (2018) 184–192

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Protective effect and mechanism of injection of glutamate into cerebellum fastigial nucleus on chronic visceral hypersensitivity in rats

Ling-ling Zhen[1], Bei Miao[1], Ying-ying Chen, Zhen Su, Man-qiu Xu, Sujuan Fei*,[2], Jianfu Zhang*,[2]

Department of Gastroenterlogy, Affiliated Hospital of Xuzhou Medical University, Xuzhou 221002, Jiangsu Province, China

ARTICLE INFO

Keywords:
Fastigial nucleus (FN)
Gamma-aminobutyric acid (GABA)
The chronic visceral hypersensitivity (CVH)
Glutamate (Glu)

ABSTRACT

Aims: We investigated the effects of chemical stimulation of cerebellum fastigial nucleus (FN) on the chronic visceral hypersensitivity (CVH) and its possible mechanism in rats.

Main methods: We stimulated the FN by microinjecting glutamate into the FN, in order to explore whether the cerebellum fastigial nucleus played a role on CVH in rat. The model of CVH was established by colorectal distension (CRD) in neonatal rats. Abdominal withdrawal reflex (AWR) scores, pain threshold, and amplitude of electromyography (EMG) were used to assess the hyperalgesia.

Key findings: We showed that microinjection of L-glutamate (Glu) into the FN markedly attenuated hyperalgesia. The protective effect of FN was prevented by pretreatment with the glutamate decarboxylase inhibitor, 3-mercaptopropionic acid (3-MPA) into the FN or $GABA_A$ receptor antagonist, bicuculline (Bic) into the LHA (lateral hypothalamic area). The expressions of protein Bax, caspase-3 were decreased, but the expression of protein Bcl-2 was increased after chemical stimulation of FN. These results indicated that the FN participated in regulation of CVH, and was a specific area in the CNS for exerting protective effects on the CVH. In addition, LHA and GABA receptor may be involved in this process.

Significance: Our findings might provide a new and improved understanding of the FN function, and might show an effective treatment strategy for the chronic visceral hypersensitivity.

1. Introduction

Irritable Bowel Syndrome (IBS) is one of the most common of all medical disorders worldwide. IBS is presently defined by a bundle of symptoms consisting mainly of recurrent abdominal pain associated with altered bowel movements [1]. Relevant epidemiological survey showed that the rate of morbidity of IBS was 10%–20% [2].

In recent years, numerous data had indicated that the mechanisms of IBS had been implicated in the pathogenesis of heredity, gender, intestinal motility disorders, visceral hypersensitivity, intestinal infections, intestinal flora imbalance, diet, mental health, functional abnormalities of the Gut-Brain Axis and so an [3]. Among them, most people thought that the chronic visceral hypersensitivity (CVH) played an important role in regulating the development of IBS.

For a long time, we believed that the cerebellum functioned only as the center of motor control. However, recent researches indicated that the cerebellar functions had greatly expanded, for example, cerebellum participated in regulations of learning, memory, cognition, language, and visceral activities [4–8]. Martner [9] had demonstrated that the electrical stimulation of FN in cats could obviously affect chewing activities, secretion of gastric acid, and the movement of the stomach, jejunum, ileum, and colon. The FN, sympathetic nerve, and vagal nerve might be involved in this process [10,11].

In a previous study, our research team had found that the microinjection of glutamate into FN markedly attenuated the stress-induced gastric mucosal damage [12], Zhu JZ et al. observed that the microinjection of $GABA_A$ receptor agonist, muscimol, into FN exacerbates stress-induced gastric mucosal damage in rats [13], Zhu SP et al. reported that the lateral hypothalamic area mediated the protective effects of microinjection of glutamate into interpositus nucleus on gastric ischemia-reperfusion injury in rats [14], Qiao X et al. demonstrated that the protective effect of histamine microinjected into cerebellar fastigial nucleus on stress gastric mucosal damage in rats [15].

An enormous amount of researches suggested that the cerebellum was not only a sub-cortical center for motor control, but also was an essential node in the integration of somatic and visceral activities. However, little has known about the role of the cerebellum in regulating chronic visceral hypersensitivity (CVH).

* Corresponding authors.
E-mail addresses: feisj99@xzmc.edu.cn (S. Fei), 100000108001@xzhmu.edu.cn (J. Zhang).
[1] Ling-ling Zhen and Bei Miao contributed equally and thus share first authorship.
[2] Jianfu Zhang and Sujuan Fei contributed equally to the study and article, thus share corresponding author.

https://doi.org/10.1016/j.lfs.2018.04.043
Received 13 February 2018; Received in revised form 17 April 2018; Accepted 24 April 2018
Available online 25 April 2018

Taken together, these pieces of evidence suggested that the cerebellum played an important role not only in sub-cortical motor center, but also in the central integration of visceral activities.

Recently, numerous neuro-anatomical studies revealed that the direct bidirectional connections between the cerebellum and hypothalamus, i.e., the hypothalamic–cerebellar projection, and the cerebellar–hypothalamic projection, which constitute the cerebellar–hypothalamic circuits [16]. The cerebellar–hypothalamic projection arises from the deep cerebellum nuclei (DCN) neurons, then, passing through the decussation of superior cerebellum peduncle (DSCP) and projecting into the lateral hypothalamic area (LHA). Meanwhile, Chen and Hillman pointed out that the three DCN are rich in GABA and glycine [17]. Furthermore, some data suggested that the GABA receptors existed in neuronal cell membrane of LHA [18], when microinjection of $GABA_A$ receptor agonist, muscimol, into LHA markedly inhibited sympathetic nerve excitability, and the discharge frequency of greater splanchnic nerve (GSN) was decreased [19]. Thus, we think the Gabaergic system may play a role in the cerebellar–hypothalamic projection.

Many information indicated that the gamma-aminobutyric acid (GABA) was the capital inhibitory neurotransmitters of the central nervous system, which possess inhibitory effects, generally on brain neurons, according to estimation, GABAergic neurons occupied above 30% approximately, in CNS. The GABA is formed by glutamate via glutamate decarboxylase (GAD). Glutamate decarboxylase is the rate-limiting enzyme for the production of γ-aminobutyric acid (GABA). But glutamate decarboxylase inhibitor, 3-mercaptopropionic acid (3-MPA), could not increase the availability and quantities of glutamate, only have a synergistic effect with glutamate. GABA receptor contains three subtypes, including $GABA_A$, $GABA_B$, and $GABA_C$ receptors. $GABA_A$ receptor antagonist, bicuculline, could suppress the effect of GABAergic neurotransmission, and then prevented the function of GABA.

All of these data indicated that the cerebellum may be one of the important sites in regulating the development of chronic visceral hypersensitivity (CVH). In the present study, we were interested in focusing on the effect and the possibly mechanism of microinjection glutamate into FN on CVH in rats.

2. Materials and methods

2.1. Reagents

The L-glutamate, bicuculline, and 3-mercaptopropionic acid (3-MPA) were obtained from Sigma-Aldrich (St. Louis, MO). Rabbit anti-Bcl-2, rabbit anti-Bax, rabbit anti-Caspase-3, rabbit anti-β-actin (Zhongshan Golden Bridge Biotech Co., Beijing, China).

2.2. Experimental animals

Newborn Sprague-Dawley (SD) rats, weighing 12–20 g were supplied by the Experimental Animal Center of Xuzhou Medical University, Xuzhou, China (usage certificate No: SYXK (su) 2002-0038). All procedures were conducted in compliance with protocols approved by the Ethics Committee of Affiliated Hospital of Xuzhou Medical University, and all the author were informed consent.

2.3. Experimental protocol

The newborn rats were randomly divided into different groups (n = 4): sham-operated group (Sham) (only use the balloon to stimulate the anus of rats), colorectal distension (CRD) group (see below), experimental groups including: L-glutamate stimulating FN (Glu) (3, 6, 12 μg L-Glu dissolved in 0.2 μl saline, respectively) + CRD group, L-glutamate decarboxylase inhibitor, 3-MPA + L-glutamate stimulating FN (12 μg L-Glu dissolved in 0.2 μl saline) + CRD group, $GABA_A$ receptor antagonist, Bicuculline + L-glutamate stimulating FN (12 μg L-Glu dissolved in 0.2 μl saline), Solvent (0.2 μl saline injection into FN or LHA, respectively) + CRD group. Rats in each group were housed in wire mesh cages with food and water available ad libitum and adapted to laboratory conditions for several days. The room temperature maintained at 23 ± 1 °C on a 12:12-hour light-dark cycle (light on at 8 am). The experimental protocol was approved by Xuzhou Medical University Animal Care and Use Committee. Prior to the experiment, all rats were fasted for 24 h, and allowed free access to tap water. The irritation procedure and the experimental testing were conducted during the light component of the cycle. The neonates were housed 8 in a cage with 1 adult female ray until they were 21 days old.

2.4. Model preparation of the chronic visceral hypersensitivity in newborn rats

According to the method of Al-Chaer et al. [20], in brief, SD rats (8 days old) in CRD group received colorectal distension on postnatal days 8, 10, and 12. The distention was applied using angioplasty balloons (OrbusNeich Inc., sapphire NC, US; length: 10.0 mm, diameter: 3.0 mm) inserted rectally into the descending colon. The balloon was distended with inflation methods, whose pressure reached 60 mm Hg (as measured with a sphygmomanometer), for 1 min, and then, deflated and withdrawn. The distention was repeated 2 times (separated by 30 min) within an hour.

2.5. Preparation of dilated balloon for adult rats

In brief, the lengths of balloons were 4 cm, approximately, and were made of the finger with a latex glove, then were attached to polyethylene tubing, and inserted through the anus into the rectum and descending colon. The open end of the balloon was secured to the tubing with thread, and wrapped with tape, to make sure there was no air leak between the balloon and the catheter. The tubing was attached via a T connector to a sphygmomanometer pump and gauge. Before they were used, the balloons were blown up and left overnight so the latex stretched and the balloons became compliant.

2.6. Behavioral testing

Behavioral responses to CRD were assessed in all groups starting 8 weeks after the cessation of the irritation protocol by measuring the abdominal withdrawal reflex (AWR) [21] using a semiquantitative score and by measuring the pain threshold of CRD that elicits an express contraction in the abdominal wall musculature.

Before the experiment, the rats were slightly anesthetized, then distention balloons (described above) were coated with paraffin oil, and placed in the descending colons of mildly sedated rats, secured by taping the attached tubing to the rat's tail. The rats were then housed in small lucite cubicles (20 cm × 12 cm × 9 cm) on an elevated plexiglass platform, and allowed to wake up and adapt (30 min). Injecting air into the balloon through a syringe, and measurement of AWR of rats response to graded CRD (20, 40, 60, 80 mm Hg) by blinded observers. The AWR was scored as follows: 0, no significant behavioral changes in rats; 1, brief head movement followed by immobility; 2, contraction of abdominal muscles; 3, lifting of abdomen; 4, body arching and lifting of pelvic structures.

Measuring the pain threshold of CRD consisted of recording the stimulus intensity that evokes a visually identifiable contraction of the abdominal wall, which was applied in increments of 10 mm Hg starting at 10 mm Hg.

For both measurements (AWR and pain thresholds), the rats were given CRD for 20 s every 4 min, and CRD in each intensity was repeated 3 times. The data for each animal were averaged for analysis.