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Role of insular cortex in visceral hypersensitivity model in rats subjected to chronic stress

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ABSTRACT

Abnormal processing of visceral sensation at the level of the central nervous system has been proven to be important in the pathophysiologic mechanisms of stress related functional gastrointestinal disorders. However, the specific mechanism is still not clear. The insular cortex (IC) was considered as one important visceral sensory area. Moreover, the IC has been shown to be involved in various neuropsychiatric diseases such as panic disorders and post-traumatic stress disorder. However, whether the IC is important in psychological stress related visceral hypersensitivity has not been studied yet. In our study, through destruction of the bilateral IC, we explored whether the IC played a critical role in the formation of visceral hypersensitivity induced by chronic stress on rats. Chronic partial restraint stress was used to establish viscerally hypersensitive rat model. Bilateral IC lesions were generated by N-methyl-D-day (door) aspartate. After a recovery period of 7 days, 14-day consecutive restraint stress was performed. The visceromotor response to colorectal distension was monitored by recording electromyogram to measure rats' visceral sensitivity. We found that bilateral insular cortex lesion could markedly inhibit the formation of visceral hypersensitivity induced by chronic stress. The insular cortex plays a critical role in the pathophysiology of stress-related visceral hypersensitivity.

1. Introduction

Functional gastrointestinal disorders (FGIDs) are characterized as chronic or recurrent gastrointestinal symptoms, which are not explained by structural or biochemical abnormalities. FGIDs are considered as a serious public health problem because they are remarkably common and bring a major social and economic burden. FGIDs mainly include irritable bowel syndrome (IBS), functional dyspepsia (FD), functional heartburn (FH), functional constipation (FC), etc. Among them, IBS is the most common with prevalence rates of 2–15% (Choung and Locke, 2011), and is characterized by recurrent abdominal pain or discomfort and disturbed bowel habits. The etiology and pathophysiology of IBS remain incompletely understood. Some factors such as psychological stress, food intolerance, intestinal infections etc. were thought to remarkably affect the symptom generation and exacerbation of IBS patients (O'Malley et al. 2011; Saha, 2014). Especially, in recent years, along with acceleration of modern life rhythm and increasing of working stress in our country, the effect of psychological stress on IBS was paid more and more attention. Visceral hypersensitivity was thought to be critical in stress related IBS (Zhou and Verne, 2011). Visceral hypersensitivity induced by psychological stress has been explored by many studies (Dunphy et al., 2003; Bian, 2012; Larauche, 2012a), and the central sensitization was thought to be critical in its genesis. In past neuroimaging studies, several cerebral regions such as anterior cingulate gyrus (ACC), prefrontal cortex (PFC) and insular cortex (IC) have been found to be abnormally activated in FGIDs (Elsenbruch et al., 2010a; Elsenbruch et al., 2010b; Zeng et al., 2011). The abnormal activities of central regions were thought to be possibly important in the generation of visceral hypersensitivity.

IC was demonstrated to be one important visceral sensory area (Yágüez et al., 2005; Bi et al., 2011). In addition, IC has been revealed to be possibly involved in a number of neuropsychiatric diseases such as mood disorders, panic disorders and post-

Abbreviations: FGIDs, functional gastrointestinal disorders; IBS, irritable bowel syndrome; FD, functional dyspepsia; FH, functional heartburn; FC, functional constipation; ACC, anterior cingulate gyrus; PFC, prefrontal cortex; IC, insular cortex; VMR, Visceral Motor Response; EMG, electromyogram; CRD, colon rectum distension; AUC/s, the area under the curve per second; NMDA, N-methyl-D-day (door) aspartate; PRS, partial restraint stress

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traumatic stress disorder (Nagai et al., 2007). Considering the roles of IC in visceral sensory and neuropsychiatric diseases, we proposed that IC may play an important role in psychological stress related visceral hypersensitivity. In present study, through destructing bilateral IC by N-methyl-D-day (door) aspartate (NMDA) in rats, we explored whether IC played a critical role in the pathophysiology of stress-related visceral hypersensitivity.

2. Materials and methods

Male Wistar rats were used in all experiments which were obtained from the Laboratory Animal Services Centre, Fudan University, China. All animals were cared at the vivarium of our institute with a range of weight between 250 and 300 g at the beginning of the experiment. Rats were maintained on a 12-h light/dark cycle and housed four per cage before the experiment. The handling of rats and all procedures performed were approved and strictly complied with the Animals (Control of Experiments) Ordinance of Tongji hospital, Shanghai, China (the ethics committee number: 2014-DW-001). N-methyl-D-day (door) aspartate was purchased from Sigma-Aldrich Inc.

2.1. Viscerally hypersensitive rat model induced by chronic stress

The stressor used in the following experiments was chronic partial restraint stress (PRS), which was described in earlier studies (Ait-Belgnaoui et al., 2006). In chronic restraint stress group, after ether anesthesia, the fore shoulders, upper forelimbs of each rat were tied to a piece of wood stick (diameter 1 cm, length 18 cm) by cotton ropes for 1 h per day on 14 consecutive days, and at 15th day EMG recording test was performed. Stress was always performed between 8:00 a.m. and 10:00 a.m. The sham stress group underwent ether anesthesia but not tied. Normal controls were without any intervention.

2.2. Bilateral IC lesions

The animals were deeply anesthetized with sodium pentobarbital (50 mg/kg). Lesions of the unilateral IC were generated by slowly microinjecting 0.3 μl N-methyl-D-day (door) aspartate solution (NMDA, Sigma Chemical Co, MO; 20 mg/ml solution in phosphate buffer 0.1 M, pH 7.4) at the rate of 0.1 μl/min at the following coordinates into the IC: anterior posterior (AP) +1.2 mm from bregma, 5.0 mm lateral to midline (L), 5.5 mm ventral to brain surface. After injection, the cannulas were kept in place for 2 min to facilitate diffusion of the drug (Ramírez-Amaya and Bermudez-Rattoni, 1999). This procedure was repeated in the contralateral insula. Sham lesions were generated using the same coordinates which received PBS (0.1 M, pH 7.4) instead of NMDA. Normal controls were without any intervention. After a recovery period of 7 days, chronic partial restraint stress was performed. The extent of IC lesions was determined by histological studies. Location of brain regions was according to charts derived from the atlas of the rat brain by Paxinos and Watson.

2.3. Visceral motor response (VMR) and electromyogram (EMG) recording in rats

To measure rats' visceral sensitivity, the VMR to colorectal distension (CRD) was monitored by recording EMG as previously described in many studies (Welting et al., 2005). Briefly, a pair of Teflon-coated stainless wires was surgically implanted into the left external abdominal oblique muscles three days before recording EMG. On the test day, animals were subject to CRD. A flexible latex balloon (medical finger glove, 4 cm long, 2.3 cm diameter flaccid) tied around a urethral catheter (3 mm diameter) was lubricated with liquid paraffin oil and inserted into descending colon with the distal tip 1 cm from the anal verge and secured to the base of the tail under short ether anesthesia. 60 mmHg CRD was performed by rapidly injecting gas into the colonic balloon over 1 s and maintaining the distention for 20 s. Three cycles of 60 mmHg CRD (20 s duration; 2 min inter-stimulus interval) were applied to each rat. The EMG recording signal was amplified and filtered (50–5000 Hz) by PowerLab System (Chart 7.0, AD Instruments, Bella Vista, NSW, Australia). The results of electromyography were quantified by calculating the area under the curve per (AUC/s). AUC is the sum of all recorded data points multiplied by the sample interval (in seconds) after baseline subtraction.

2.4. Histological analysis

After completing the EMG recording, the brains were removed and fixed. The sections were stained with Nissls staining and analyzed to assess the extent of the damage caused by the lesions.

2.5. Statistical analyses

All data were expressed as mean ± S.D.. statistical analysis was performed using SPSS 17.0. Comparison between three groups was constructed by one-way ANOVA test and within groups by LSD test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Comparison of visceral sensitivity after chronic stress

Comparing among normal control (NC), sham-PRS and chronic PRS groups, there were significant differences in AUC/s ($P < 0.001$, Fig.1A). The mean AUC/s was significantly enhanced in chronic PRS group, but no markedly differences between sham-PRS and normal control groups ($P=0.940$, Fig.1A). Representative electromyographic recordings for 60 mmHg rectal distension in normal control (NC), sham-PRS and chronic PRS groups are shown in Fig.1B.

3.2. Comparison of visceral sensitivity after IC lesion

Compared with PRS group and sham-lesion+PRS group, the AUC/s markedly decreased in IC-lesion+PRS group. There were no significant differences between sham-lesion+PRS and PRS groups ($P=0.059$, Fig.2 A). Representative electromyographic recordings for 60 mmHg rectal distension in PRS, sham-lesion+PRS and IC-lesion+PRS groups are shown in Fig.2B.

3.3. Insular cortex lesion

In our study, bilateral IC lesions were generated by microinjection of NMDA. The histological analysis showed that bilateral IC lesions were mainly located in the medial part of the anteroposterior axis of the IC (Fig.3), which was consistent with prior research of Ramírez-Amaya and Bermudez-Rattoni. (Ramírez-Amaya and Bermudez-Rattoni, 1999). Representation photomicrograph of NMDA-induced lesions into the insular cortex is showed in Fig. 3C. Fig. 4 shows serial reconstructions of the largest and smallest lesions in the insular cortex induced by NMDA. The rats with inadequate lesion (i.e. lesions located outside the target area or with no apparent lesions) were discarded from our study according to the previous study (Ramírez-Amaya et al., 1998). Those inadequate lesion rats comprised less than 10% of the total number of animals.