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Role of adrenomedullin in the cerebrospinal fluid-contacting nucleus in the modulation of immobilization stress

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ABSTRACT

The contribution of the cerebrospinal fluid-contacting nucleus (CSF-contacting nucleus) and adrenomedullin (ADM) to the developmental modulation of stressful events remains controversial. This study explored the effects of endogenous ADM in the CSF-contacting nucleus on immobilization of stress-induced physiological parameter disorders and glucocorticoid hormone releasing hormone (CRH), rat plasma corticosterone expression, and verification of such effects by artificially lowering ADM expression in the CSF-contacting nucleus by targeted ablation of the nucleus. Immunohistochemical experiments showed that ADM-like immunoreactivity and the calcitonin receptor-like receptor (CRLR) marker were localized in the CSF-contacting nucleus. After 7 continuous days of chronic immobilization stress (CIS), animals exhibited anxiety-like behavior. Also, an increase in serum corticosterone, and enhanced expression of ADM in the CSF-contacting nucleus were observed, following activation by CIS. The intracerebroventricular (i.c.v.) administration of the ADM receptor antagonist AM22–52 significantly reduced ADM in the CSF-contacting nucleus, additionally, blocked the effects of ADM, meaning the expression of CRH in the hypothalamic paraventricular nucleus (Pa) and serum corticosterone level were increased, and the physiological parameters of the rats became correspondingly deteriorated. Additionally, the i.c.v. administration of cholera toxin subunit B-saporin (CB-SAP), a cytotoxin coupled to a cholera toxin subunit, completely eliminated the CSF-contacting nucleus, worsening the reaction of the body to CIS. The collective results demonstrated that ADM acted as a stress-related peptide in the CSF-contacting nucleus, and its lower expression and blocked effects in the nucleus contributed to the deterioration of stress-induced physiologic parameter disorders as well as the excessive expressions of stress-related hormones which were part of the hypothalamic–pituitary–adrenal (HPA) axis.

1. Introduction

The CSF-contacting nucleus is a special nucleus found in the brain, which was initially discovered and named by our group worldwide (Lu et al., 2008; Wang and Zhang, 1992; Zhou et al., 2013). This nucleus is composed of special neurons called the distal cerebrospinal fluid-contacting neurons (dCSF-CNs) whose bodies are in the parenchyma and whose processes extend into CSF in the cavity, distributing and localizing in the ventral periaqueductal central gray (PAG) of the brainstem. Such a unique anatomical structure means that the dCSF-CNs can bidirectionally transmit signals between the brain parenchyma and CSF by absorbing or releasing bioactive substances, a role that would not be shared by any other nucleus in the brain (Zhang et al., 2003). However, the biological role of this nucleus is unclear, and researchers have studied the subject for more than 20 years. Previous studies demonstrated that the CSF-contacting nucleus contributed to noise stress (Wang et al., 2007) in rats and was involved in the regulation of pain (Chao et al., 2010; Lu et al., 2009; Wang et al., 2014), but the basic materials in such a nucleus and its possible role and mechanisms involved in stress are unclear.

Adrenomedullin (ADM) is a 52-amino-acid peptide in humans belonging to the calcitonin gene related peptide (CGRP) superfamily and was initially isolated from pheochromocytoma in 1993 (Kitamura et al., 1993). ADM and its receptors have wide distribution (Hwang and Tang, 2000; Serrano et al., 2000) and multiple actions in many systems such as the central nervous system (Kuwasako et al., 2011). The potent actions of ADM within the brain have been demonstrated in several studies (Cheung and Tang, 2012; Oyar et al., 2011; Shan and Krukoff, 2001; Sugimoto et al., 2013).

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Since its discovery, numerous studies have reported that ADM acts at each level of the hypothalamic–pituitary–adrenal (HPA) axis (Shan and Krukoff, 2001). The i.c.v. administration of exogenous ADM can influence the expressions of stress related hormones of the HPA axis (Samson et al., 1995; Troughton et al., 2001), but its actions at the individual autonomic centers in the brain are heterogeneous, since these effects are the opposite of those resulting from its i.c.v. administration (Allen et al., 1997). Also, the i.c.v. administration of ADM led to elevated plasma corticosterone levels in rats. This effect was abrogated by pretreatment with a CRH antagonist (Taylor and Samson, 2004), but the central effects of ADM on the HPA axis were the opposite of its direct effects on pituitary cells. These results suggested that exogenous ADM activated the HPA axis, which led to the excessive expressions of stress-related hormones and stress-related pathophysiological changes. The process appears to be quite complex, though, leaving many contradictory phenomena which require further study. However, many recent studies have demonstrated that ADM has protective functions under various stressful conditions such as traumatic (Armstead et al., 2010), acidosis (Wu et al., 2007), and ischemic stress (Kataoka et al., 2010), and these functions mainly display themselves in the inhibition of inflammation (Li et al., 2005; MacManus et al., 2011), oxidative stress (Wang and Yang, 2009), apoptosis (Xia et al., 2006) and excitotoxicity (Fujita et al., 2005). In this case, the role of ADM underlying stress remains contradictory. Studies examining stress hormone secretion during blocked action or decreased production of brain-derived ADM are needed to confirm the role of central ADM in the physiological response to stress.

It was hypothesized that the role of the CSF-contacting nucleus in stress may be related to the regulation of stress neurocircuitry and endogenous stress-related neurochemicals. The present study examined the presence of ADM as well as its receptor in the CSF-contacting nucleus, its expression changes underlying immobilization stress, and the effects of artificially dampening and blocking the CSF-contacting nucleus effects of ADM on physiologic parameters and the expression levels of stress-related hormones of the HPA axis.

2. Materials and methods

2.1. Animals and groups

Male Sprague–Dawley rats (240–260 g, grade SPF) were provided by the Experimental Animal Center of Xuzhou Medical College (license number: SYXK 2002–0038). The animals were maintained under controlled temperatures and light cycles (23 ± 1 °C, 12/12 h dark/light cycle with lights on at 8:00 am) in protected units for at least 7 days prior to the experiments. All experimental protocols were performed based on the approval of the Animal Care and Use Committee of Xuzhou Medical College (Xuzhou, Jiangsu Province, China). All treatments and behavioral monitoring were done in a balanced design to avoid order and time discrepancies. To develop this study, the rats were divided into eight groups as follows: (1) normal; (2) CIS; (3) normal + saline; (4) normal + AM22–52; (5) CIS + saline; (6) CIS + AM22–52; (7) CIS + CB-SAP; and (8) CIS + (CB + SAP) (n = 6 for all groups).

2.2. Drugs and treatments

Rats in the CIS group were given CIS for seven continuous days. On the fifth day of CIS, a 3 μl volume of 30% CB-HRP (Sigma, USA) was injected into one of the lateral ventricles of the rats in the normal and CIS groups. AM22–52 (Sigma, USA) was dissolved in saline and administered i.c.v. at a dose of 20 μg on the last day to the normal + AM22–52 and CIS + AM22–52 groups. Targeted ablation of the CSF-contacting nucleus was performed using CB-SAP (San Diego, CA, USA), which was dissolved in artificial cerebrospinal fluid (ACSF) and administered i.c.v. at a dose of 500 μg/3 μl 7 days before the CIS procedure for the CIS + CB-SAP group. The compound of CB and saporin (900 ng/3 μl and 500 ng/3 μl, respectively) acted as a control of CB-SAP and was given to the CIS + (CB + SAP) group. After that, rats were given CIS for seven continuous days. The doses of CB-SAP and AM22–52 were chosen based on previous studies from our group and other researchers.

2.3. CIS procedure

The attraction for using the CIS model was due to the fact that it is simple, effective, and rarely if ever involves any bodily harm to the animal subjects. This ensures that any long-term effects of stress observed are due to the stressor which was applied rather than to the physical repercussions of an irreversible or chronic injury (Grandin, 2002). CIS protocol was adapted from the improved previous procedure (Al-Mohaisen et al., 2000). Rats of stressed groups were bound 1 h/d for 7 constant days. The bandage was folded from the junction of the abdomen and thighs and then fixed to something to make sure the posterior limbs were off the ground and the two forelimbs touched the ground. It was tight enough that the rats could move freely but not escape, and the limbs were not ischemic or injured. The experiments were processed between 8:00 and 11:00 am, compliant with the circadian corticotropic rhythm. In the CIS + CB-SAP and CIS + (CB + SAP) groups, CIS was given on the seventh day after the drugs were administered. Non-stressed groups were kept without food and water during the entire period of exposure to stress.

2.4. Intracerebroventricular administration

Before CIS, rats of relative groups were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and then immobilized in the Digital Lab Standard Stereotaxic Apparatus (Stoelting, USA). Drugs were injected slowly into one of the lateral ventricles of the rats according to stereotaxic coordinates (Budantsev et al., 1993) (Brega: 1.2 ± 0.4 mm, deep: 3.2 ± 0.4 mm, right to median sagittal plane: 1.4 ± 0.2 mm) over a 15 min period using a 10 μl Hamilton microsyringe needle, which was kept in place for a further 15 min to prevent drug diffusion.

2.5. Double immunostaining and imaging

All double immunostaining procedures were operated following the previous research report. Briefly, the CSF-contacting nucleus sections (40 μm) were incubated in the following antisera: purified rabbit polyclonal anti-rat ADM (1:100; Santa Cruz, USA), rabbit polyclonal anti-CRLR (1:100; Santa Cruz, USA), and goat polyclonal anti-CB-HRP (1:400; Sigma, USA). The antibodies to Cholera Toxin and ADM or CRLR were visualized with a 2 h incubation in a cocktail containing donkey anti-goat IgG-FITC (1:200; Life Technologies, USA) and donkey anti-rabbit IgG-FITC (1:200; Life Technologies, USA) in PBST. Finally, the sections were rinsed, mounted, and coverslipped. Tissue sections were examined using laser scanning confocal microscopy (TCS SP2, Leica, Germany) to identify the CSF-contacting nucleus, ADM, and CRLR.

2.6. Measurement of physiologic parameters

Locomotor activity was measured after the immobilization procedure on the last day of the model in the open-field paradigm, as previously described (Budni et al., 2007). The blood pressure and heart rate of rats were measured during the immobilization procedure by the tail-cuff method with Softron BP98A (Softron Beijing Biotechnology Co. Ltd., China). The body weight of rats before and after CIS was measured between 8:00 and 11:00 h. The proportion