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The role of calcitonin gene-related peptide in post-stroke depression in chronic mild stress-treated ischemic rats

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HIGHLIGHTS

- Chronic mild stress following stroke leads to post-stroke depression in male rats.
- CUMS-treated ischemic animals have increased CGRP levels in hippocampus.
- Central infusion of CGRP into ischemic rat induces PSD in a dose-dependent manner.
- Central infusion of CGRP antagonist produced antidepressant effects in PSD rats.

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ABSTRACT

Poststroke depression (PSD) is the most common psychological sequel after stroke. Although the neurological mechanisms of PSD remain to be fully elucidated, numerous studies have implicated the calcitonin gene-related peptide (CGRP), a potent vasodilatory neuropeptide, as key modulator of the depression. A PSD rat model, which was established by middle cerebral artery occlusion (MCAO) and following chronic unpredictable mild stress (CUMS) procedures, was used to investigate the role of CGRP in post-stroke mood disturbances. In the present study, depressive-like state such as anhedonia and behavioral despair was found in CUMS-treated ischemic rat, as measured by sucrose preference test, open-field test and forced swimming test. Moreover, CGRP immunoreactivity (CGRP-ir) concentration in CSF and hippocampus were increased in the PSD rats, compared to the MCAO or CUMS subjects. The other separate groups were implanted chronically with unilateral cannulae in the lateral cerebral ventricle. GABA and its receptor antagonist $\alpha GABA_{8-37}$ were administrated centrally into ischemic and PSD rats, respectively. Administration of CGRP into the ischemic rat increased depression-like behaviors in a dose-dependent manner, whereas icv infusion of $\alpha CGRP_{8-37}$ produced antidepressant effects in PSD rats, implying that the PSD is mediated, at least partially, by endogenous CGRP receptor activation. Taken together, these results suggest a pivotal role for central CGRP signaling in the modulation of PSD.

1. Introduction

Poststroke depression (PSD) is the most common psychological sequel of stroke, which occurs approximately one-third of all stroke survivors [1]. Depression following stroke is associated with increased morbidity and mortality [2], poorer functional recovery and quality of life [1,3], as well as higher risk of recurrent stroke [4]. Although there have been numerous studies focused on its epidemiological features and outcomes [5–7], the pathophysiology of PSD remains to be fully elucidated. Some studies suggest that depression after stroke is a psychological reaction to functional disability, whereas others propose a biological mechanism related stroke-induced brain lesion and presumably subsequent changes in neurotransmitters [6]. Despite the fact that a high proportion of stroke patients develop PSD, underlying neurobiological mechanisms have only sparsely been investigated. An animal model that capitulates these biological and psychosocial causes and effects would be ideal for the PSD research, providing the major prerequisite for deeper insights into the biological basis of post-stroke mood disturbances.

The hippocampus, a prominent limbic brain structure, has been implicated in many aspects of the neuroendocrine responses during stress, including emotional responses [8,9]. In animals, lesions or inactivation of the hippocampus reduces stress and depressive-like behavior in several paradigms [10]. It is also supported by many neuroimaging studies in humans. Alterations in the levels of neurotransmitters as monoamines and their receptors in the hippocampus and adjacent temporal lobe have been demonstrated to be critical in the modulation of depressive behavior. Moreover, neuropeptides and neurotrophins

Abbreviations: MCAO, Middle cerebral artery occlusion; CUMS, Chronic unpredictable mild stress; PSD, Poststroke depression; CGRP, Calcitonin gene-related peptide; CSF, Cerebrospinal fluid

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(such as brain-derived neurotrophic factor, BDNF) have also been advanced as important modulators of depression, based on analyses of knockout and transgenic mouse models, as well as human genetic studies.

Calcitonin gene-related peptide (CGRP), a 37-amino-acid neuropeptide, best known for its potent vasodilatory properties [8,11,12], has been implicated in many aspects of the depression. Anatomically, CGRP has been costored and in part coreleased with classical depressive neurotransmitters in hippocampus [13,14]. Moreover, functional studies support that CGRP may contribute to the regulation of many psychological and behavioral processes, such as stress responses, anorectic, addictive and fear-related behaviors [15–18]. Furthermore, clinical research indicates that CGRP levels in cerebrospinal fluid (CSF) [19,20] are altered in subjects suffering from major depressive disorder. More specifically, CGRP-immunoreactive (CGRP-ir) in the hippocampus and frontal cortex is elevated in "genetically depressed" Flinders Sensitive Line rat, while maternal separation (a behavioral model of depression) could even exacerbate the elevation [21]. Consistently, a recent study shows that, through methylation of the CGRP gene, gestational environment programs adult depression-like behavior [22]. These results strongly indicate that genetic disposition and development stress may contribute to the susceptibility to depression, at least partially, by exerting CGRP-specific effects on adult neurobiology.

Although PSD is as a subtype of vascular depression, its symptom and treatment-response profiles appear to be more similar to major depression [23,24]. Indeed, some underlying biological mechanisms, such as the amine hypothesis, may play a role in both depression disorder and PSD. Nevertheless, whether CGRP participate in mood disorders following stroke has not been investigated. To test this hypothesis, PSD animal model was established by combining middle cerebral artery occlusion (MCAO) and chronic unpredictable mild stress (CUMS) treatment procedure. The involvement of CGRP in the process of PSD was investigated by examining the levels of CGRP-ir in the central nervous system in PSD rats. Furthermore, we investigated whether intracerebroventricular (icv) administration of CGRP would induce depressive-like states in ischemic rats and central injection of CGRP receptor antagonist $CGRP_{8\text{-}37}$ would attenuate depressive-like behaviors in PSD rats.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats, weighing 250–270 g, obtained from Wenzhou Medical University, were housed under controlled conditions (12 h:12 h light–dark, with lights on at 0700 h; temperature at 22 ± 2 °C) and provided with food and water *ad libitum*. To investigate the changes of CGRP concentrations in the central nervous system following the ischemic stroke and stress, animals were randomly assigned into four groups in experiment 1: (1) sham-operated rats (n = 9); (2) MCAO rats (n = 9); (3) sham-operated rats treated with CUMS (n = 9); (4) MCAO rats treated with CUMS (n = 9). The other sets of rats were used in experiment 2 involving the drug administration: (1) MCAO rats treated with vehicle (n = 8); (2) MCAO rats treated with different dose of CGRP (each n = 8); (3) PSD rats treated with vehicle (n = 8); (4) PSD rats treated with CGRP antagonist (n = 8). All animal procedures were performed in accordance with the Guidelines of the Chinese Council on Animal Care and approved beforehand by the Institutional Animal Care and Use Committee of Wenzhou Medical University. All surgical procedures were carried out under ketamine (100 mg/kg ip; Pharmacia and Upjohn, Crawley, UK) and Rompun (10 mg/kg ip; Bayer, Leverkusen, Germany) anesthesia.

2.2. Middle cerebral artery occlusion model

Transient focal cerebral ischemia was induced by 90 min of left middle cerebral artery (MCA) followed by reperfusion as described previously [25], with minor modifications. Sham animals underwent the same procedure except that the MCA was not occluded. Cerebral blood flow reduction of 80% of baseline after the MCAO was confirmed in ischemic animals by Laser Doppler Flowmetry (Moor Instruments).

2.3. CUMS procedures

After a 7-day recovery period following the MCAO, the CUMS procedure was performed on animals as described previously [26], with minor modifications. The procedure contained nine different stressors randomly arranged day and night across 14 consecutive days: 18 h water deprivation, 20 h food and water deprivation, 12 h of 45° cage tilt, 21 h wet cage, overnight illumination, 2 min swimming in water at 4 °C, 2 min swimming in water at 45 °C, 1 min tail pinch and 2 h immobilization.

2.4. Brain cannula implantation

For experiment 2 involving the central administration of CGRP or its receptor antagonist, groups of rats were fitted with unilateral icv guide cannula (22 gauge; Plastic One, USA) positioned towards the left lateral cerebral ventricle, the co-ordinates for implantation being 0.6 mm lateral, 1.5 mm posterior to Bregma, and 4 mm below the surface of the dura [27]. The guide cannula was secured using dental cement (Dental Filling Ltd., UK), and fitted with a dummy cannula (Plastics One) to maintain patency. Brain cannulae were implanted just after the CUMS procedures.

2.5. Behavioral assessments

Sucrose preference test (SPT) and open-field test (OFT) were employed to determine the establishment of the PSD model, while SPT and forced swimming test (FST) were used to investigate the effects of drugs on PSD state in rats.

2.5.1. Sucrose preference test

The SPF was used to operationally determine anhedonia. In the SPT which was conducted between 0900 h and 1000 h, the animals were allowed to consume water and 1% sucrose solution for 1 h after 20 h food and water deprivation. The sucrose preference index was calculated according to the following ratio: sucrose preference = sucrose intake (g)/sucrose intake (g) + water intake (g). A baseline preference test was performed before the CUMS procedure, and the sucrose preference was monitored during periods of modeling and treatment.

2.5.2. Open-field test

The OFT was performed to evaluate general locomotor and rearing activity of rats. The apparatus consisted of a dark varnished wooden box (100 cm square chamber, 40 cm high walls) with the floor divided into 25 equal squares. Locomotor activity was defined as at least three paws in a quadrant and rearing behavior defined as the animal standing upright on its hind legs were tallied over a 3-min period.

2.5.3. Forced swimming test

The FST conducted as described by Detke et al. [28]. Briefly, rats were placed individually for 15 min in a glass cylinder (50 cm tall and 20 cm in diameter) containing tap water to 35 cm depth (25 ± 1 °C). Twenty-four hours after their first exposure (training purposes, with no data collected), the animals were replaced in the swim apparatus for 5 min, and the session was recorded by two trained observers. Climbing behavior consisted of upward movements of the forepaws in and out of the water, while immobility was assigned when the rat remained afloat in the water without additional activity other than that required to keep the rat's head above the water.