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Alarin-induced antidepressant-like effects and their relationship with hypothalamus–pituitary–adrenal axis activity and brain derived neurotrophic factor levels in mice

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ABSTRACT

Alarin is a newly identified member of the galanin family of peptides. Galanin has been shown to exert regulatory effects on depression. Similar to galanin in distribution, alarin is also expressed in the medial amygdala and hypothalamus, i.e., regions interrelated with depression. However, it remains a puzzle whether alarin is involved in depression. Accordingly, we established the depression-like mouse model using behavioral tests to ascertain the possible involvement of alarin, with fluoxetine as a positive control. With the positive antidepressant-like effects of alarin, we further examined its relationship to HPA axis activity and brain-derived neurotrophic factor (BDNF) levels in different brain areas in a chronic unpredictable mild stress (CUMS) paradigm. In the acute studies, alarin produced a dose-related reduction in the immobility duration in tail suspension test (TST) in mice. In the open-field test, intracerebroventricular (i.c.v.) injection of alarin (1.0 nmol) did not impair locomotion or motor coordination in the treated mice. In the CUMS paradigm, alarin administration (1.0 nmol, i.c.v.) significantly improved murine behaviors (FST and locomotor activity), which was associated with a decrease in corticotropin-releasing hormone (CRH) mRNA levels in the hypothalamus, as well as a decline in serum levels of CRH, adrenocorticotropic hormone (ACTH) and corticosterone (CORT), all of which are key hormones of the HPA axis. Furthermore, alarin upregulated BDNF mRNA levels in the prefrontal cortex and hippocampus. These findings suggest that alarin may potentiate the development of new antidepressants, which would be further secured with the identification of its receptor(s).

Introduction

Depression is a common debilitating disorder which can be triggered by chronic psychosocial stress, with well-characterized etiological factors [48] in vulnerable individuals [12,22]. With more populations affected than ever before, depression is becoming one of the most widespread health threats to humans. However, current antidepressants available have many limitations [4], such as low efficacy (i.e., approximately one-third of patients responded to the agents initially prescribed), and there is a delay ranging from several weeks to months until a therapeutic effect is observed. Therefore, it is essential to discover new targets and alternative antidepressants in depression treatment.

Neuropeptides are considered as the largest class of neuromessengers in the central nervous system (CNS), and are induced by high-frequency neuronal activity. In recent years, more attentions are paid to the role of neuropeptides in depression [5,37]. The galanin family of peptides is a pleiotropic group with numerous functions. Several studies indicate a role for galanin family in depression [24,38,45]. For instance, galanin is distributed at high levels in the hypothalamus and amygdale, which have been implicated in depression [6,15]. Likewise, galanin is involved in the stress response, and galanin agonist or galanin receptor subtype 3 antagonists have antidepressant-like effect [31,32]. There are reports that galanin plays significant roles in the regulation of depression-like behaviors via their antagonism against hyperactivity of hypothalamus–pituitary–adrenal (HPA) axis [41,57]. Given the well-characterized roles of galanin in mood

Abbreviations: GALP, galanin-like peptide; HPA, hypothalamus–pituitary–adrenal; FST, forced swim test; TST, tail suspension test; CUMS, chronic unpredictable mild stress; CORT, corticosterone; ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; BDNF, brain derived neurotrophic factor; mpFC, medial prefrontal cortex; CNS, central nervous system; aCSF, artificial cerebrospinal fluid; FLU, fluoxetine; GC, glucocorticoid.

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disorders, we investigated the effects of alarin on depression-like behaviors and the possible underlying mechanism.

Alarin is identified as the third member of galanin family, initially in gangliocytes of human neuroblastic tumors [46] and was regarded as an alternate transcript of the GALP gene [27,46,47]. To date, evidence is limited regarding the physiological role or pharmacological properties of alarin. It was shown that in the periphery, alarin has the anti-edema effect due to its function as a vasoconstrictor [47]. A recent study showed that alarin was detected in various ocular tissues and its involvement in ocular health was confirmed [50]. In CNS, intercerebroventricular (i.c.v.) injection of alarin dose-dependently increased acute food intake [3,58] and body weight. Alarin also affects luteinizing hormone secretion in rats. More recently, alarin is observed to be highly expressed in the CNS tumors, such as ependymoma and choroid plexus tumors [10]. Nowadays, its antibacterial effect has been identified [59]. However, the other functions in the peripheral and CNS still remain obscure. It would be of interest to reveal its new physiological activity on depression.

Researches have shown that alarin is widely distributed throughout the entire murine brain, including the mitral cell layer of the olfactory bulb, accessory olfactory bulb, different nuclei of the hypothalamus such as the arcuate nucleus and the ventromedial hypothalamic nucleus, and the locus coeruleus [9]. Hypothalamus and the locus coeruleus are acknowledged as key regions in mood-related disorders. Meanwhile, galanin has been reportedly to exhibit effects on depression. However, whether alarin also has antidepressant-like effect becomes our focus of attention.

Hyperactivity of the HPA axis and disturbance of stress feedback are common features in major depression. There are clinical findings that the levels of adrenocorticotropic hormone (ACTH), corticosteroids (cortisol in human and corticosterone in animals, CORT) were elevated in patients with depression, and at the end of treatment with antidepressants, the levels of plasma ACTH and CORT were reduced [13,19,20].

In recent years, brain-derived neurotrophic factor (BDNF) is also a hot topic in the research of depression. In terms of neurotrophic factor theory, depression is generally accompanied by downregulated BDNF in the brain, following antidepressant treatment however, the expression of BDNF was upregulated in rodent brain as well as human serum [8,29]. The majority of antidepressants can upregulate BDNF mRNA in the brain, and the upregulated endogenous BDNF thereby reverses neuronal atrophy and leads to cell proliferation in the hippocampus and prefrontal cortex, with depression symptoms attenuated.

Still, it remains a puzzle whether alarin alleviates the depression-like behaviors in mice is dependent on the inhibited hyperactivity of HPA axis and the increased level of BDNF. The purpose of our study was to observe the effects of alarin on depressive-like behaviors in mouse models of both acute and chronic stress-induced depression and to clarify the underlying mechanisms, and to further explore the relationship between the antidepressant-like effect and HPA axis and BDNF.

Experimental procedures

Animals

Male C57BL/6J mice, weighing 18–22 g upon arrival, were individually housed at a constant temperature (23 ± 2 °C) and maintained on a 12 h/12 h light/dark cycle (lights on from 8:00 am to 20:00 pm) with ad libitum access to food and water. All mice were acclimatized for at least 1 week prior to experiment. All experiments in the present study were complied with the Chinese Council on Animal Care and Institutional Care Committee of Xuzhou Medical College.

Drugs

Full-length alarin (APAHRSSPFPPRPTRAGRETQLLRS; alarin 1–25, drug purity of 96.39%) was custom-synthesized (GL Biochem, Shanghai, China) and freshly prepared in artificial cerebrospinal fluid (aCSF, Gibco, Grand Island, NE, U.S.A.). Mice were lateral ventricularly injected (i.c.v.) with 0.1, 0.5, 1.0 or 2.0 nmol synthetic alarin. Fluoxetine hydrochloride (FLU) (Sigma–Aldrich, Saint Louis, MO, U.S.A.) was dissolved in aCSF for administration in each mouse at 10 nmol prior to the experiment. The aCSF consisted of 0.13 mol/l NaCl, 0.98 mmol/l $MgCl_2$, 2.65 mmol/l KCl, 1.2 mmol/l $CaCl_2$, 0.25 mmol/l ascorbic acid and 10 mmol/l glucose dissolved in double-distilled water (DDW).

Intracerebroventricular injections

Intracerebroventricular injections were performed in the left ventricle with a procedure as described by Haley and McCormick [16]. The injection needle (external diameter of 0.52 mm) of a Hamilton microsyringe (10 μl) fitted with a stainless stop to attain a depth of 3.5 mm was inserted from the skull. The coordinates for microinjections were 2.0 mm lateral to the bregma, with a total injection volume of 3 μl. Injections were performed 50 min prior to the behavioral tests by a technician blinded to the experiments.

Chronic unpredictable mild stress (CUMS)

Male C57BL/6J mice were subjected to a variety of mild stressors as previously described [11,34] with minor modifications. The following stimuli were administered each week in a random order, for six weeks: 3 h at a 45° cage tilt, twice a week; moisture for 12 h, once a week, water deprivation for 14 h, once a week; lights on for 9 h during the dark phase, once a week; noise for 3 h, thrice a week, and flashing light for 30 min, thrice a week. The decrease in the responsiveness to rewarding stimuli in mice was monitored by consumption of sucrose solution. Apart from the effects of CUMS on the sucrose preference, other behavioral parameters are essential, e.g., changes in exploratory/locomotor behavior, variations in body weight and durations in FST.

Behavioral analysis

In the acute experiments, prior to the behavioral tests, each mouse underwent respective intracerebroventricular injection of aCSF, alarin or FLU. 50 min afterwards, behavioral tests were conducted. Mice underwent open-field test (OFT) for 5 min, immediately followed by tail suspension test (TST).

In the chronic experiments, with the depression-like models successfully established, a single injection (i.c.v.) of aCSF or alarin or FLU was performed as scheduled in each mouse for sucrose preference test (24 h). On day 4, the injection was repeated in each mouse. 50 min afterwards, behavioral tests including OFT and forced swim test (FST) were conducted.

Forced swim test

FST for mice was modified from a previous protocol [42]. Briefly, mice were transferred into a transparent plastic cylinder (20 cm in diameter × 35 cm in height) with water of 20 cm in depth at 23–25 °C. The immobility duration was recorded. The immobility was defined as the absence of all movements with the exception of motions required to maintain the murine head above the water. Mice were individually tested, and the duration of immobility was recorded for the last 4 min of the 6-min testing period, i.e., after