Archival Report

Biological Psychiatry

Ablation of Type III Adenylyl Cyclase in Mice Causes Reduced Neuronal Activity, Altered Sleep Pattern, and Depression-like Phenotypes

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ABSTRACT

BACKGROUND: Although major depressive disorder (MDD) has low heritability, a genome-wide association study in humans has recently implicated type 3 adenylyl cyclase (AC3; *ADCY3*) in MDD. Moreover, the expression level of AC3 in blood has been considered as a MDD biomarker in humans. Nevertheless, there is a lack of supporting evidence from animal studies.

METHODS: We employed multiple approaches to experimentally evaluate if AC3 is a contributing factor for major depression using mouse models lacking the *Adcy3* gene.

RESULTS: We found that conventional AC3 knockout (KO) mice exhibited phenotypes associated with MDD in behavioral assays. Electroencephalography/electromyography recordings indicated that AC3 KO mice have altered sleep patterns characterized by increased percentage of rapid eye movement sleep. AC3 KO mice also exhibit neuronal atrophy. Furthermore, synaptic activity at cornu ammonis 3–cornu ammonis 1 synapses was significantly lower in AC3 KO mice, and they also exhibited attenuated long-term potentiation as well as deficits in spatial navigation. To confirm that these defects are not secondary responses to anosmia or developmental defects, we generated a conditional AC3 floxed mouse strain. This enabled us to inactivate AC3 function selectively in the forebrain and to inducibly ablate it in adult mice. Both AC3 forebrain-specific and AC3 inducible knockout mice exhibited prodepression phenotypes without anosmia.

CONCLUSIONS: This study demonstrates that loss of AC3 in mice leads to decreased neuronal activity, altered sleep pattern, and depression-like behaviors, providing strong evidence supporting AC3 as a contributing factor for MDD.

Keywords: Ciliopathies, Major depressive disorder (MDD), Neuronal activity, Primary cilia, Sleep alteration, Type III adenylyl cyclase (AC3, *ADCY3*)

http://dx.doi.org/10.1016/j.biopsych.2015.12.012

Major depressive disorder (MDD) affects approximately 17% of the US population and is one of the most common debilitating disorders, with lifetime prevalence of ~15% (1–3). MDD has a complex and heterogeneous nature and is considered to be the outcome of gene–environmental interactions (2,4). Studies using mouse models have suggested that depression may result from loss of synapses and correspondingly altered neuronal activity in limbic and cortical regions (5–8). Current pharmacologic treatments of depression rely on elevating monoamine concentrations in the brain (1–3). Interestingly, most antidepressants, including selective serotonin reuptake inhibitors, have the potential to indirectly stimulate adenylyl cyclase activity through G-protein coupled adrenergic and serotonin receptors. Indeed, a number of studies have implicated adenylyl cyclase activity in depression (9), and platelet adenylyl cyclase activity has been proposed as a biological marker for MDD (10,11). This is based on the fact that subjects with a history of depression have lower mean levels of platelet adenylyl cyclase activity compared with control subjects. The type 3 adenylyl cyclase (AC3) was thought to be a major isoform of adenylyl cyclases in human platelets (12). AC3 is also the predominant adenylyl cyclase in primary cilia throughout the central nervous system (CNS) (13,14). It is widely recognized that primary cilia are the cellular antenna for most of vertebrate cells including central neurons. Neuronal primary cilia do not have synaptic structure and largely depend on metabotropic receptors and downstream signal proteins to execute their functions. In this sense, AC3 is a key mediator of the cyclic adenosine monophosphate (cAMP) signaling in primary cilia of the brain.

MDD is familial but has low heritability compared with other major psychiatric disorders (2,6,7,15). However, a recent study based on thousands of patients with MDD and healthy subjects has implicated cAMP signaling in depression and identified AC3 (*ADCY3*) as a top-ranked gene relevant for MDD (16). Consistently, depressed patients have decreased

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 ISSN: 0006-3223

transcript levels of AC3 in blood (17). Therefore, we examined the role of AC3 in depression using transgenic mice lacking AC3. We found that conventional AC3 knockout (KO) mice exhibited depression-like behaviors. Moreover, experimental evidence from conditional knockout mice also support AC3 as a gene associated with depression.

METHODS AND MATERIALS

The Supplement includes detailed Supplemental Methods and Materials.

Mice

AC3 KO mice and AC3 wild-type (WT) littermates were bred from heterozygotes and genotyped as previously reported (18). The mice used in behavioral analysis were age-matched 2.5- to 4-month-old male mice with comparable body weight, if not otherwise indicated. Mice were maintained on a 12-hour light/dark cycle at 22°C and had access to food and water ad libitum.

Sholl Analyses of Morphology of Primarily Cultured Cortical Neurons From AC3 WT or KO Mice

Sholl analyses of neuronal morphology were performed as previously described with some modifications (19). Cortical neurons were isolated and cultured from AC3 WT or AC3 KO newborn pups at postnatal day 1, as previously described (20).

Whole-Cell Blind-Patch Recording of Pyramidal Neurons in Cornu Ammonis 1 Region

Hippocampal slices were cut from young AC3 WT and AC3 KO mice (4–6 weeks) under ice-cold high-magnesium sucrose solution: 87 mmol/L sodium chloride, 2.5 mmol/L potassium chloride, 7 mmol/L magnesium chloride, .5 mmol/L calcium chloride, 1.25 mmol/L monosodium phosphate, 25 mmol/L sodium bicarbonate, 10 mmol/L D-glucose, and 75 mmol/L sucrose. After cutting, slices were placed in artificial cerebrospinal fluid to recover for 1 to 2 hours at room temperature. Whole-cell mode was achieved using the blind patch method (21).

RESULTS

AC3 KO Mice Demonstrate Depression-like Behaviors

AC3 KO mice were subjected to a number of behavioral assays for depression including the tail suspension test, forced swim test, sociability test, novelty-suppressed feeding, and nesting behavior. In the tail-suspension test, AC3 KO mice demonstrated significantly longer periods of immobility than their AC3 WT littermates (Figure 1A). Similarly, AC3 KO mice exhibited longer periods of immobility in the forced swim test than AC3 WT mice (Figure 1B). Furthermore, AC3 KO mice spent much less time interacting with a target mouse compared with AC3 WT mice in a three-chamber sociability test (Figure 1C). When feeding in a novel environment, AC3 KO mice were much slower to feed than AC3 WT mice (Figure 1D), and their total feeding time in a novel environment was much less than AC3 WT mice (Figure 1E). When examined for drinking behavior in a novel environment, AC3 KO mice were slower to begin drinking (Figure 1F), and their total drinking time was markedly reduced (Figure 1G). AC3 KO mice also had lower coat score compared with AC3 WT mice, another marker for depression. Moreover, AC3 KO mice also exhibited defects in nesting behavior. Although they prepared nests in their home cages, their nests were poorly constructed compared with AC3 WT mice (Figure 1H, I). When placed in a novel environment, which posed mild stress to mice, the nest-building behavior of AC3 KO mice compared with AC3 WT mice was more impaired (Figure 1J, K). Collectively, these data indicate that AC3 KO mice exhibit behaviors consistent with depression.

AC3 KO Mice Spend More Time in Rapid Eye Movement Sleep

Disturbances of sleep including alterations in sleep architecture and increased rapid eye movement (REM) sleep are typical for MDD patients and are one of the core symptoms associated with MDD (22,23). Therefore, the sleep architecture of AC3 KO mice was analyzed by electroencephalography (EEG)/electromyography recordings. During a 24-hour period, AC3 KO mice spent approximately the same amount of time awake as AC3 WT mice (Figure 2A, B). However, AC3 KO mice spent significantly more time than AC3 WT mice in REM sleep during the 24-hour light/dark period (Figure 2A, C). Throughout the circadian cycle, AC3 KO mice spent approximately twice as much time in REM sleep as AC3 WT mice (Figure 2C). Moreover, AC3 KO mice demonstrated altered nonrapid eye movement (NREM) sleep patterns compared with WT littermates (Figure 2D). In addition, The NREM sleep wave was less synchronized in AC3 KO mice (Figure 2E, F). EEG power analysis of NREM sleep wave revealed that the peak power (power at peak frequency) and the total delta power (.5–4 Hz) of NREM sleep wave of AC3 KO mice were markedly reduced compared with AC3 WT mice (Figure 2F–H). These data indicate that AC3 KO mice have altered sleep patterns and sleep less efficiently than AC3 WT mice.

Loss of AC3 Causes Neuronal Atrophy

Major depression is associated with neuronal atrophy in the CNS, which is centered in the hippocampus (8). We therefore compared the brain size and found that the brain of $AC3^{-/-}$ mice is smaller than WT mice (Figure 3A). The weight of the $AC3^{-/-}$ mouse brain is markedly lighter than $AC3^{+/+}$ mice (Figure 3B). We also measured the bregma-lambda distance of $AC3^{-/-}$ and $AC3^{+/+}$ mice using a stereotaxic system as an indicator of brain size. The bregma-lambda distance of $AC3^{-/-}$ mice was significantly shorter than $AC3^{+/+}$ mice (Figure 3C). We further examined if the hippocampal volume of AC3 KO mice is decreased. Indeed, AC3 KO mice have smaller hippocampi than their littermate control mice (Figure 3D). Stereological analysis of AC3 KO brain sections further revealed that both areas cornu ammonis (CA)1 (Figure 3E) and the dentate gyrus (Figure 3F) of the hippocampus are significantly smaller than wild-type littermates. These data indicate that AC3 KO mice have reduced volume of hippocampi than their WT littermates.

AC3 is found in primary cilia in pyramidal neurons of the hippocampus and is implicated in dendritic arborization (19). Therefore, we compared the morphology of primarily cultured cortical neurons from AC3 KO mice with those from AC3 WT mice (Figure 3G). Sholl analysis demonstrated that cultured