Behavioural Brain Research 307 (2016) 1–10

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Studies of mice with cyclic AMP-dependent protein kinase (PKA) defects reveal the critical role of PKA's catalytic subunits in anxiety

George Briassoulis[a,b], Margaret F. Keil[a,*], Bilal Naved[a], Sophie Liu[a], Matthew F. Starost[c], Maria Nesterova[a], Nirmal Gokarn[a], Anna Batistatos[a], T.John Wu[d], Constantine A. Stratakis[a]

[a] Section on Endocrinology and Genetics, Program in Developmental Endocrinology and Genetics, Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH), Bethesda, MD 20892, United States
[b] Department of Pediatric Intensive Care, University of Crete, Heraklion, Greece
[c] Division of Veterinary Resources, Office of Research Services (ORS), Office of the Director (OD), National Institutes of Health, Bethesda, MD 20892, United States
[d] Department of Obstetrics and Gynecology and Center for Neuroscience and Regenerative Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, United States

HIGHLIGHTS

- Alterations of total PKA activity in the amygdala regulate the anxiety response.
- Both R1α and Cα subunit activity regulate PKA signaling in the amygdala and anxiety.
- Cα activity is not the sole determinant of PKA's cAMP signaling effects.

ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form 23 February 2016
Accepted 1 March 2016
Available online 16 March 2016

Keywords:
Anxiety
Amygdala
Protein kinase a
Knockout mice

ABSTRACT

Cyclic adenosine mono-phosphate-dependent protein kinase (PKA) is critically involved in the regulation of behavioral responses. Previous studies showed that PKA's main regulatory subunit, R1α, is involved in anxiety-like behaviors. The purpose of this study was to determine how the catalytic subunit, Cα, might affect R1α's function and determine its effects on anxiety-related behaviors.

The marble bury (MB) and elevated plus maze (EPM) tests were used to assess anxiety-like behavior and the hotplate test to assess nociception in wild type (WT) mouse, a *Prkar1a* heterozygote *(Prkar1a*$^{+/-}$*)* mouse with haploinsufficiency for the regulatory subunit (R1α*)*, a *Prkaca* heterozygote *(Prkaca*$^{+/-}$*)* mouse with haploinsufficiency for the catalytic subunit *(Cα)*, and a double heterozygote mouse (*Prkar1a*$^{+/-}$/*Prkaca*$^{+/-}$) with haploinsufficiency for both R1α and Cα. We then examined specific brain nuclei involved in anxiety.

Results of MB test showed a genotype effect, with increased anxiety-like behavior in *Prkar1a*$^{+/-}$ and *Prkar1a*$^{+/-}$/*Prkaca*$^{+/-}$ compared to *WT* mice. In the EPM, *Prkar1a*$^{+/-}$ spent significantly less time in the open arms, while *Prkaca*$^{+/-}$ and *Prkar1a*$^{+/-}$/*Prkaca*$^{+/-}$ mice displayed less exploratory behavior compared to *WT* mice. The loss of one *Prkar1a* allele was associated with a significant increase in PKA activity in the basolateral (BLA) and central (CeA) amygdala and ventromedial hypothalamus (VMH) in both *Prkar1a*$^{+/-}$ and *Prkar1a*$^{+/-}$/*Prkaca*$^{+/-}$ mice.

Alterations of PKA activity induced by haploinsufficiency of its main regulatory or most important catalytic subunits result in anxiety-like behaviors. The BLA, CeA, and VMH are implicated in mediating these PKA effects in brain.

Published by Elsevier B.V.

1. Introduction

In brain, genetic manipulations of cAMP-dependent protein kinase A (PKA) suggest its critical role in a wide spectrum of neurobiological and psychiatric disorders. The PKA is a multi-substrate

* Corresponding author at: National Institutes of Health, NICHD/PDEGEN, 10 Center Drive, Building 10 CRC, Room 1 E3330, Bethesda, MD 20892, United States.
E-mail address: keilm@mail.nih.gov (M.F. Keil).

http://dx.doi.org/10.1016/j.bbr.2016.03.001
0166-4328/Published by Elsevier B.V.

serine/threonine protein kinase responsible for modulating a variety of cellular processes [1–4]. It is a heterotetramer composed of two N-terminally dimerized regulatory (R) subunits bound to two catalytic (C) subunits [5,6]. PKA activation involves simple subunit dissociation upon binding of cAMP to the R subunits; thereby freeing catalytically active C subunits, which in turn phosphorylate protein substrates that contain appropriate consensus phosphorylation motifs [7]. One target of PKA is the cAMP responsive element (CRE) binding protein (CREB). Phospho-CREB in turn binds to CREs to mediate gene expression that has been linked to numerous behavioral reactions including those to stressors [8–10]. The response generated by cAMP can be terminated by the hydrolysis of cAMP into 5′AMP by phosphodiesterases (PDEs). Various studies indicate that increased cAMP signaling is associated with an anxiety-like phenotype and suggest that elevated cAMP activity is associated with abnormal reactivity to novel environments [11,12], stress-coping responses [13,14], and an overall increased anxiety [15–18]. Thus, therapeutic manipulation of PKA activation, such as decreasing phosphorylation of cAMP–CREB might lead to new treatment strategies for anxiety, addiction, or psychiatric disorders [3,19–21].

Our laboratory has studied the consequences of R and C subunit dysregulation [22]. The *Prkar1a* and *Prkaca* genes encode the type 1A regulatory (R1α) and type A catalytic (Cα) subunits of PKA [23]. Regulatory subunits form a homodimer that binds two catalytic subunits (Cα, Cβ, or Cγ coded by the *PRKACA*, *PRKACB*, and *PRKACG* genes, respectively) in the PKA tetramer (R2C2) [8]. When the R and C subunits form a complex, the cAMP-catalytic activity is suppressed. As shown in knockout (KO) mouse studies, these four genes function in a tissue and cell-type specific manner to regulate the activity of the catalytic subunits [5]

Recently, we reported that loss of one *Prkar1a* allele in mice (*Prkar1a*$^{+/-}$) led to an augmentation of anxiety-like behaviors in association with an increase in PKA activity in both the basolateral (BLA) and central amygdala (CeA) [24]. These regions of the amygdala coordinate fear- and anxiety-like behavioral responses [24–27] as well as external cues, responding to threats via direct and indirect projections to the paraventricular nucleus of the hypothalamus (PVH) and brainstem regions [27–29]. PKA activity is increased in *Prkar1a*$^{+/-}$ mice because of a deficiency in overall Cα inhibition, although the net effect varies widely in different tissues [25–30].

We reasoned that the introduction of half-null alleles of Cα into the *Prkar1a*$^{+/-}$ mice might abrogate the excess Cα activity caused by R1α haploinsufficiency. Thus, we crossed *Prkar1a*$^{+/-}$ and *Prkaca*$^{+/-}$ mice to generate double heterozygous *Prkar1a*$^{+/-}$/*Prkaca*$^{+/-}$ mice and expected a reversal of the anxiety phenotype that we observed previously [24,27] in *Prkar1a*$^{+/-}$ mice, consistent with what has been reported in PKA activity regulation [25,26]. The data perhaps not unexpectedly support attenuation but not elimination of the anxiety phenotype noted in *Prkar1a*$^{+/-}$ knockout mice (given the variable expression of other PKA subunits in brain) and highlight the importance of even modest changes in total PKA activity in the regulation of anxiety behaviors and the fact that Cα is not the sole determinant of PKA's cAMP signaling effects.

2. Materials and methods

2.1. Animals

All mice were housed three to four per standard barrier cages on a ventilated rack in a room with a constant temperature (~22+/−1 °C) with same-sex littermates with *ad libitum* access to food and water and maintained on a 12:12 light schedule (lights on at 0600 h). All animals were adults at the time of testing (2–7 months old at time of behavioral testing; 6- 10 months at time of biochemical testing). Throughout the entire experimental period, the mice were handled daily and weighed weekly to acclimate to the investigator. All animal procedures were conducted in accordance with the standards approved by the NIH Guide for the Care and Use of Laboratory Animals. All animal protocols received prior approval at the NIH. All behavioral testing was performed, as previously reported [31–33], between the hours of 1300–1700 h. One behavioral test per day was performed, with a span of at least two days between tests. The order of behavioral tests was randomly assigned [27]. Two scorers performed behavioral testing and obtained scoring of all results in a blinded fashion (without knowledge of the genotypes of the mice under observation).

2.2. Generation of *Prkar1a*±/−/*Prkaca*±/− double heterozygous mice

Prkar1a heterozygous mice (*Prkar1a*$^{+/-}$) with one null allele of *Prkar1aΔ2* were previously generated in our laboratory [28]. *Prkaca* heterozygous mice (*Prkaca*$^{+/-}$), which have a neomycin-resistance cassette to replace exons 6–8 of the *Prkaca* gene [29], were purchased from Mutant Mouse Regional Resource Centers (MMRRC) (strain name: B6; 129 × 1-Prkaca*tm1Gsm*/Mmnc). We used heterozygotes *Prkaca* mice because the majority of Cα knockouts are either embryonic lethal or not viable and die in the immediate postnatal period [29,34]. In addition, using heterozygotes for both *Prkar1a*$^{+/-}$ *and Prkaca*$^{+/-}$, and also double heterozygotes, we ensured equivalent genotyped groups, more appropriate for multiple comparisons. *Prkar1a*$^{+/-}$ *and Prkaca*$^{+/-}$ mice were crossed to generate the *Prkar1a*$^{+/-}$/*Prkaca*$^{+/-}$ double heterozygous mice, which were maintained on a mixed C57BL/6 129 Sv/B6 hybrid background (REF). All mice were genotyped using tail DNA. Control (wild-type, *WT*) mice were used from the same litter and were matched for age. Genotyping was conducted by polymerase chain reaction (PCR) using primers previously validated [30]. Three primers (5′-AGCTAGCTTGGCTGGACGTA-3′, 5′-AAGCAGGCGAGCTATTAGTTTAT-3′ and 5′-CATCCATCTCCTATCCCCTTT-3′) were used for *Prkar1a* genotyping: the WT allele generated a 250 base pair (bp) fragment and the null allele generated an180 bp product. Primers and conditions for the PCR reactions are available upon request [28,35]. Genotyping of *prkaca* was done with two pairs of primers: the first pair (5′- CTGACCTTTGAGTATCTGCAC-3′ and 5′- GTCCCACACAAGGTCCAAGTA-3′) was used to detect the *WT* allele by amplification of the intron between exons 6 and 7 with a product of 250 bp; the second pair was used to detect the knockout allele (5′- AGACTACTGCTCTATCACTGA-3′ and 5′-GTGGTTTGTCCAAACTCATCAATGT-3′) by amplification of a 270-nucleotide fragment of the region between the 3′-end of the neomycin resistance gene and a portion of the intron just 3′ to exon 8. The wild-type allele generated a 6.0-kb fragment, and the knockout allele generated a 2.7-kb fragment.

2.3. Mouse phenotyping, numbers, behavioral tests, necropsies

Adrenal tumors or corticosterone overproduction were not identified in *Prkar1a*$^{+/-}$ or *Prkar1a*$^{+/-}$/*Prkaca*$^{+/-}$ mice by the age they were used in this study; likewise, thyroid neoplasms, schwannomas and other tumors were observed only in older *Prkar1a*$^{+/-}$ mice, as reported previously [28]. No mice with tumors were used in this study. In addition, standard assessment of neurological function [24] revealed no deficits in mice enrolled in the study. A total of 55 control (wild-type, *WT*), 40 *Prkar1a*$^{+/-}$, 29 *Prkaca*$^{+/-}$, and 33 *Prkar1a*$^{+/-}$/*Prkaca*$^{+/-}$ mice (all littermates) were handled for the purposes of this project.