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Inhibition of phosphodiesterase 2 reverses impaired cognition and neuronal remodeling caused by chronic stress

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ABSTRACT

Chronic stress and neuronal vulnerability have recently been recognized as factors contributing to cognitive disorders. One way to modify neuronal vulnerability is through mediation of phosphodiesterase 2 (PDE2), an enzyme that exerts its action on cognitive processes via the control of intracellular second messengers, cGMP and, to a lesser extent, cAMP. This study explored the effects of a PDE2 inhibitor, Bay 60-7550, on stress-induced learning and memory dysfunction in terms of its ramification on behavioral, morphologic, and molecular changes. Bay 60-7550 reversed stress-induced cognitive impairment in the Morris water maze, novel object recognition, and location tasks (object recognition test and/or object location test), effects prevented by treatment with 7-NI, a selective inhibitor of neuronal nitric oxide synthase; MK801, a glutamate receptor (NMDAR) inhibitor; myr-AIP, a CaMKII inhibitor; and KT5823, a protein kinase G inhibitor. Bay 60-7550 also ameliorated stress-induced structural remodeling in the CA1 of the hippocampus, leading to increases in dendritic branching, length, and spine density. However, the neuroplasticity initiated by Bay 60-7550 was not seen in the presence of 7-NI, MK801, myr-AIP, or KT5823. PDE2 inhibition reduced stress-induced extracellular-regulated protein kinase activation and attenuated stress-induced decreases in transcription factors (e.g., Elk-1, TORC1, and CREB phosphorylation) and plasticity-related proteins (e.g., Egr-1 and brain-derived neurotrophic factor). Pretreatment with inhibitors of NMDA, CaMKII, neuronal nitric oxide synthase, and protein kinase G (or protein kinase A) blocked the effects of Bay 60-7550 on cGMP or cAMP signaling. These findings indicate that the effect of PDE2 inhibition on stress-induced memory impairment is potentially mediated via modulation of neuroplasticity-related NMDAR-CaMKII-cGMP/cAMP signaling.

1. Introduction

The major factors involved in age-related neurodegenerative diseases, such as progressive decline in cognitive functions and the loss of synaptic contacts, remain poorly understood. There is significant evidence indicating that chronic stress and neuronal vulnerability are interrelated events contributing to age-related pathologies, such as Alzheimer's disease (AD) (Xu et al., 2009). In response to sustained stress, the brain undergoes a complex array of cellular and molecular changes that lead to maladaptive remodeling which presents itself in ways such as learning and memory impairment (McEwen , 2000; Mitra and Sapolsky, 2008). Presently, a plethora of different molecular "culprits" have been linked to architectural changes of neurons during chronic stress. NMDA receptor (NMDAR)-mediated Ca^{2+} influx is a major cellular mechanism for synaptic plasticity and learning and memory and may affect cyclic AMP (cAMP) and/or cyclic GMP (cGMP) formation through Ca^{2+}-calmodulin-dependent adenylyl cyclase, and neuronal nitric oxide synthase (nNOS) related guanylyl cyclase (Huang et al., 1994; Chen et al., 2010). Cyclic AMP and cGMP are 2 likely candidates contributing to intracellular signaling transduction and gene transcription in the process of learning and memory (Burns et al., 1996; Suvarna and O'Donnell,

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2002). The dysregulation of cAMP and/or protein kinase A (PKA) and cGMP and/or protein kinase G (PKG) signaling in states of prolonged stress reduces neuronal firing and rapidly affects cognitive function through disruption of neuroplasticity-associated proteins and their upstream transcriptional regulators (Birnbaum et al., 2004; Bodhinathan et al., 2010; Hains et al., 2009).

The main therapeutic option when regulating cAMP or cGMP is through intervention of phosphodiesterase (PDEs), which are a superfamily of enzymes that regulate cAMP and cGMP by hydrolysis. The discovery of phosphodiesterases inhibitors brings hope for revealing the processes underlying stress-induced cognitive deficits and uncovering strategies for the treatment of age-related disorders, such as AD. There are at least 11 distinct PDEs, and their inhibitors have been shown to be involved in intracellular signaling pathways associated with neurodegenerative disorders or their treatment (Xu et al., 2011). One of the particular interest within the central nervous system is phosphodiesterase 2 (PDE2), primarily because of the high expression in the limbic nervous system and the adrenal cortex (Van Staveren et al., 2003), areas associated with cognitive functions and hypothalamus-pituitary-adrenal axis regulation. Expression in these key areas helps promote the idea that PDE2 plays a role in the pathogenesis of stress-related disorders, including learning and memory impairment (Reneerkens et al., 2009). However, it is not clear whether PDE2 inhibitors contribute to architectural changes in the hippocampus or how they affect the processes of learning and memory by regulation of series of molecular events when animals are subjected to unpredictable stress.

The present study was conducted to determine whether the PDE2 inhibitor Bay 60-7550 could ameliorate the structural remodeling of hippocampal neurons and related memory changes observed following chronic stress. The findings will help provide evidence that PDE2 inhibitors can modulate neuroplasticity and cognitive function by linking NMDAR-CaMKII-cGMP/cAMP signaling to downstream synaptic proteins expression.

2. Methods

2.1. Animals

Male ICR mice (Harlan, Indianapolis, IN, USA) weighing between 22 and 25 g (3 months old) at the start of the experiment were obtained from the Animal Center of West Virginia University. Mice were housed 5 per cage under standard colony conditions, with a 12-hour light and/or dark cycle and access to food and water ad libitum. All experiments were carried out according to the "NIH Guide for the Care and Use of Laboratory Animals" (NIH Publications No. 80-23, revised 1996). Experimental procedures were approved by the Animal Care and Use Committee of West Virginia University Health Sciences Center.

2.2. Drugs and treatments

Bay 60-7550 (2-[(3,4-dimethoxyphenyl)methyl]-7-[(1R)-1-hydroxyethyl]-4-phenylbutyl]-5-methyl-imidazo[5,1-f][1,2,4]triazin-4(1H)-one) and KT5823 (2,3,9,10,11,12-hexahydro-10R-methoxy-2,9-dimethyl-1-oxo-9S, 12R-epoxy-1H-diindolo[1,2,3-fg:3′,2′,1′-kl]pyrrolo[3,4-i][1, 6]benzodiazocine-10-carboxylic acid, methyl ester) were obtained from Cayman Chemical (Ann Arbor, MI, USA). Myristoylated autocamtide-2-related inhibitory peptide (myr-AIP), MK801, and H89 were purchased from Calbiochem (San Diego, CA, USA). 7-Nitroindazole (7-NI) and Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME) were purchased from Sigma Aldrich.

Bay 60-7550 was dissolved in 0.5% dimethyl sulfoxide and was administered via the intraperitoneal route (i.p.). myr-AIP, MK801, KT5823, and H89 were dissolved in artificial cerebrospinal fluid. Bay 60-7550 (1 and 3 mg/kg) or vehicle was given 30 minutes before stress procedures once per day for 14 days. MK801 (10 μM), myr-AIP (20 μM), 7-NI (20 mg/kg), L-NAME (20 mg/kg), KT5823 (20 μM), and H89 (5 μM) were administered 30 minutes before treatment with Bay 60-7550. Animals were given bilateral microinjections of 2 μL MK801 and myr-AIP (1 μL/side) into the CA1 of the hippocampus. All the behavioral tests were performed 24 hours after last drug treatment.

2.3. Surgery for brain cannula implantation

Animals were anesthetized intraperitoneally with a ketamine and/or xylazine mixture (100 mg/kg and 10 mg/kg, i.p., respectively) and placed in a stereotaxic frame (Stoelting Instruments, USA) with flat-skull position. Two holes are drilled on the skull based on the coordinates for hippocampal CA1 (AP −1.7 mm from bregma, ML ±0.8 mm from midline, and DV −2.0 mm from dura) (Franklin and Paxinos, 1997) before a guide cannula (30 gauge) was inserted in each hole and fixed in place. The cannula was anchored to the skull with dental cement, and then stainless steel stylets were inserted into the guide cannula to maintain patency before microinjections and to prevent occlusion. All surgery was performed under aseptic conditions. The mice were allowed to recover for 7–10 days.

2.4. Chronic unpredictable stress

Chronic unpredictable stress paradigm comprises exposure of mice to 2 different stressors twice daily for 14 consecutive days according to the procedure described earlier with minor modification (Ortiz et al., 1996; Perrotti et al., 2004; Willner et al., 1992). The order of stressors used was as follows: day 1, shaker stress (high speed, 45 minutes), cold water swim (12 °C, 5 minutes); day 2, restraint stress (1 hour), tail pinch (1 minute); day 3, food and/or water deprivation (6 hours), 6-hour social isolation (the mice were placed individually in different cage in another housing room and were returned to their home cage 6 hours later); day 4, cold water swim (12 °C, 5 minutes), lights on overnight; day 5, cage tilting (6 hours), shaker stress (high speed, 1 hour); day 6, tail pinch (1 minute), food and/or water deprivation (6 hours); day 7, cold room (4 °C, 15 minutes), 6-hour social isolation; day 8, shaker stress (high speed, 1 hour), restraint (1 hour); day 9, switching cages (6 hours); lights on overnight; day 10, cage tilting (6 hours), cold water swim (12 °C, 5 minutes); day 11, 6-hour social isolation, tail pinch (1 minute); day 12, humid sawdust (6 hours), food and/or water deprivation (6 hours); day 13, cold room (4 °C, 15 minutes), switching cages (6 hours); day 14, lights on overnight, cage tilting (6 hours). This protocol has been shown to cause significant effects on a number of cellular, biochemical, and neurochemical parameters characteristic of depressive and/or anxiogenic behaviors (Ortiz et al., 1996; Perrotti et al., 2004; Willner et al., 1992). Control groups were also handled everyday and kept in their home cages for the 14-day period. Three sets of mice were used for experiments including Morris water maze (MWM), novel object recognition, and novel object location tests, each of which had 10 groups of mice.

2.5. Morris water maze

The apparatus consisted of a circular, plastic pool (95 cm diameter × 25 cm high) located in a well-illuminated room with external cues visible from the inside of the pool, which was filled with opaque water (21 ± 1 °C). A hidden circular platform