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Protein kinase C activity is associated with prefrontal cortical decline in aging

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Abstract

Aging is associated with deficiencies in the prefrontal cortex, including working memory impairment and compromised integrity of neuronal dendrites. Although protein kinase C (PKC) is implicated in structural plasticity, and overactivation of PKC results in working memory impairments in young animals, the role of PKC in prefrontal cortical impairments in the aged has not been examined. This study provides the first evidence that PKC activity is associated with prefrontal cortical dysfunction in aging. Pharmacological inhibition of PKC with chelerythrine rescued working memory impairments in aged rats and enhanced working memory in aged rhesus monkeys. Improvement correlated with age, with older monkeys demonstrating a greater degree of improvement following PKC inhibition. Furthermore, PKC activity within the prefrontal cortex was inversely correlated with the length of basal dendrites of prefrontal cortical neurons, as well as with working memory performance in aged rats. Together these findings indicate that PKC is dysregulated in aged animals and that PKC inhibitors may be useful in the treatment of cognitive deficits in the elderly.

Keywords: Prefrontal cortex; Aging; Working memory; Protein kinase C; Dendritic spines; Chelerythrine; Dendrites

1. Introduction

Studies of aged animals and humans show prominent deficits in prefrontal cognitive functions including working memory (Albert, 1997; Ando and Ohashi, 1991; Bartus et al., 1978; Bimonte et al., 2003; Moore et al., 2006, 2005; Nielsen-Bohlman and Knight, 1995; Rapp and Amaral, 1989; Schacter et al., 1996; West, 1996). Impairments in these executive operations compromise the regulation of thought, emotion and behavior, and ultimately jeopardize independence and quality of life. Prefrontal cortical cognitive operations have become especially important in the information age, when one must steer through a constant barrage of distractions even to perform simple tasks. Elucidating mechanisms underlying prefrontal cortical decline is critical for the treatment of debilitating cognitive deficits in the elderly.

Goldman-Rakic (1995) described working memory microcircuits as local networks, comprised of pyramidal cells engaged in recurrent excitation, creating persistent firing, and GABAergic interneurons, which provide spatial tuning. Prefrontal cognitive impairments may stem from disruptions to the structural components of such microcircuits (Liston et al., 2006). Studies of humans and animals have established that advanced aging is associated with reduced neuropil (Uylings and de Brabander, 2002) including reduced

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dendritic arborizations and spine density of prefrontal cortical pyramidal cells (Cupp and Uemura, 1980; Duan et al., 2003; Harmon and Wellman, 2003; Jacobs et al., 1997; Nakamura et al., 1985; Page et al., 2002; Peters et al., 1998; Scheibel et al., 1975; Uemura, 1980; Uylings et al., 2000) as well as 40–50% reduction in synapse density in superficial layers of the prefrontal cortex (Peters et al., 2001, 1998). Thus, the anatomical substrates of prefrontal networks are affected in the aged. These changes are likely reflected in the loss of prefrontal gray matter, which has been reported in aged humans (Gunning-Dixon and Raz, 2003; Raz et al., 1997).

Protein kinase C (PKC) comprises a family of kinases that regulate of a variety of nervous system functions, including cell–cell communication, cytoskeletal reorganization and neuronal housekeeping via phosphorylation of myriad substrates. More than 10 isoforms of PKC have been identified, and the majority of these isoforms are present within the brain (reviewed in Battaini (2001)). PKC isoforms are classified based on cofactors required for activation. Calcium-dependent (conventional) isoforms require both free calcium and diacylglycerol (DAG) for activation. Calcium-independent (novel) isoforms require DAG only, and atypical isoforms are activated independent of DAG and calcium (Battaini, 2001; Battaini and Pascale, 2005). Both calcium-dependent and calcium-independent PKC isoforms regulate the actin cytoskeleton (Larsson, 2006) and overactivation of PKC results in spine loss and altered spine morphology *in vitro* (Calabrese and Halpain, 2005) suggesting a role for PKC overactivation in structural deficits in the aged prefrontal cortex. PKC also plays a role in prefrontal cognitive deficits.

Overactivation of PKC via Gq receptor stimulation produces working memory impairments in young animals (Birnbaum et al., 2004). This signaling cascade results in increased activation of PKCα as well as increased total PKC activity within the prefrontal cortex (Birnbaum et al., 2004), suggesting additional isoforms may also be activated. Accordingly, PKC inhibitors targeting a wide array of PKC isoforms improve working memory performance and reverse working memory impairments in rats and monkeys (Birnbaum et al., 2004; Runyan et al., 2005).

Although no studies have examined PKC activation within the aged prefrontal cortex, studies of the hippocampus (Colombo and Gallagher, 2002; Colombo et al., 1997) and the entire cortex (Battaini et al., 1990, 1995; Battaini and Pascale, 2005) indicate that the cellular distribution and quantity of activated PKC is altered in the aged brain. Studies relating PKC and cognitive status in the aged have focused largely on the hippocampus. PKC is crucial for hippocampal memory formation (Mathis et al., 1992; Paylor et al., 1991; Serrano et al., 1994; Yang and Lee, 1993; Zhao et al., 1994) and alterations in PKCγ contribute to deficits in hippocampal mediated memory in the aged (Barnes et al., 1988; Battaini et al., 1990, 1995; Colombo and Gallagher, 2002; Colombo et al., 1997; Gianotti et al., 1993). It is important to recognize that the hippocampus and the prefrontal cortex are regulated differently and therefore knowledge accumulated from studies of the hippocampus cannot be assumed to generalize to the prefrontal cortex.

The present study sought to clarify the relationship between activation of PKC and structural and functional integrity of the aged prefrontal cortex. We hypothesized that cognitive impairments in the aged involve dysregulation of PKC in the prefrontal cortex. Since structural changes are pronounced in the aged, we also examined dendritic length and spine density within layer III prefrontal pyramidal neurons, the putative site of the network interactions that maintain information over a delay. Our findings indicate that working memory performance and dendritic integrity are inversely related to the activity of PKC in the aged prefrontal cortex, and that inhibition of PKC activity may be useful in the treatment of prefrontal impairments in the elderly.

2. Materials and methods

2.1. Working memory performance and PKC inhibition in non-human primates

Thirteen female rhesus monkeys (*Macaca mulatta*) ranging in age from 18 to 35 years were individually housed and maintained on a diet of Purina monkey chow (St. Louis, MO). All animal procedures were approved by the Yale Animal Care and Use Committee and were in accordance with the National Institute of Health's Guide for Care and Use of Laboratory Animals.

The monkeys were trained and tested on the spatial delayed response task as described previously (Arnsten et al., 1988). Briefly, the animal watched as an experimenter baited one of 2–4 wells with a food reward. The total number of wells varied depending on the monkey's performance level. After baiting, wells were covered with identical plaques, and an opaque screen was lowered for a delay. Following the delay, the screen was raised and the animal was allowed to choose one well. Delays were titrated as described previously (Arnsten et al., 1988) such that each monkey maintained a baseline performance of 60–73% correct.

Chelerythrine chloride (CHEL, LC Laboratories, Woburn, MA) inhibits PKC activation by blocking the site of DAG/phorbol-ester binding and by inhibiting PKC translocation to the membrane for activation. Oral administration of 30 μg/kg CHEL has been previously shown to reverse working memory impairments following administration of the α-1 noradrenergic receptor antagonist, cirazoline, which activates PKC via the Gq signaling cascade (Birnbaum et al., 2004). Pilot studies indicated that higher doses of CHEL impair performance. Thus, the current study examined the effects of doses at or below the 30 μg/kg dose. Monkeys were orally administered 0.3, 3.0 or 30.0 μg/kg of CHEL dissolved in water or vehicle (water) 1 h prior to testing by a single experimenter who was unaware of the drug treatment conditions. All animals received all treatments conditions, with at least 7 days between treatments.