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Caffeine increases mitochondrial function and blocks melatonin signaling to mitochondria in Alzheimer's mice and cells

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ABSTRACT

Caffeine and melatonin have been shown to protect the Swedish mutant amyloid precursor protein (APP_{sw}) transgenic mouse model of Alzheimer's disease from cognitive dysfunction. But their mechanisms of action remain incompletely understood. These Alzheimer's mice have extensive mitochondrial dysfunction, which likely contributes to their cognitive decline. To further explore the mechanism through which caffeine and melatonin protect cognitive function in these mice, we monitored the function of isolated mitochondria from APP_{sw} mice treated with caffeine, melatonin, or both in their drinking water for one month. Melatonin treatment yielded a near complete restoration of mitochondrial function in assays of respiratory rate, membrane potential, reactive oxygen species production, and ATP levels. Caffeine treatment by itself yielded a small increase in mitochondrial function. However, caffeine largely blocked the large enhancement of mitochondrial function provided by melatonin. Studies with N2a neuroblastoma cells stably expressing APP_{sw} showed that specific inhibition of cAMP-dependent phosphodiesterase (PDE) 4 or cGMP-dependent PDE5 also blocked melatonin protection of mitochondrial function, but A_{2a} and A_1 adenosine receptor antagonists were without effect. Melatonin or caffeine at the concentrations used to modulate mitochondrial function in the cells had no effect on cAMP-dependent PDE activity or cellular cAMP or cGMP levels. Therefore, caffeine and increased cyclic nucleotide levels likely block melatonin signaling to mitochondria by independent mechanisms that do not involve adenosine receptor antagonism. The results of this study indicate that melatonin restores mitochondrial function much more potently than caffeine in APP_{sw} transgenic mouse and cell models of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is characterized by decreased cognitive function, which is largely caused by decreased synaptic function in the hippocampus and cerebral cortex regions of the brain. At the molecular level, Alzheimer's disease is characterized by mitochondrial dysfunction (Santos et al., 2010a), altered calcium signaling (Wang and Sun, 2010), and decreased axonal transport (Stokin and Goldstein, 2006). Histopathological hallmarks of AD include extracellular amyloid plaques and intracellular tangles consisting of hyperphosphorylated tau protein. However, it is still unclear the extent that each of these neurochemical and neuropathologic markers contribute to the progression of the disease.

The mitochondrial dysfunction present in Alzheimer's disease is likely caused, in part, by oligomers of Aβ interacting with mitochondrial proteins or phospholipids (Crouch et al., 2005; Kagan and Thundimadathil, 2010). However, there is also evidence of mitochondrial dysfunction caused by full length APP (Hansson Petersen et al., 2008), hyperphosphorylated tau (Eckert et al., 2010), or truncated apolipoprotein A4 (ApoE4) (Chen et al., 2011). Since mitochondrial dysfunction may originate from one of many

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different sources in the cell, antioxidants that stabilize mitochondrial respiratory function and decrease mitochondrial production of reactive oxygen species (ROS) have been pursued as therapies for AD treatment. This approach is supported by the findings that the antioxidant peptide SS31, mitoQ (Calkins et al., 2011; Manczak et al., 2010), alpha-lipoic acid (Quinn et al., 2007), Coenzyme Q10 (Chaturvedi and Beal, 2008), the natural polyphenols resveratrol (Kim et al., 2007), curcumin (Frautschy et al., 2001; Ishrat et al., 2009), and green tea epigallocatechin gallate (EGCG) (Rezai-Zadeh et al., 2008), as well as other combinations of antioxidants (Parachikova et al., 2010), can slow the rate of or reverse cognitive dysfunction in Alzheimer's rodent models. However, some antioxidants have been shown to decrease oxidative damage without preventing cognitive dysfunction (Siedlak et al., 2009). Therefore in addition to acting as antioxidants, the beneficial compounds may be activating neuroprotective signaling pathways in the brain or be acting as mitochondrial enhancers.

The hormone melatonin has been shown to be a potent antioxidant with neuroprotective effects in AD mice and other AD models (Cheng et al., 2006; Olcese et al., 2009). Melatonin may also protect against AD by preventing aggregation of Aβ monomers into oligomers (He et al., 2010; Pappolla et al., 1998). In our own work along this line, we have found that long-term oral melatonin treatment prevents memory impairment in AD mice and that this protection is associated with decreased brain Aβ deposition due to the profound anti-aggregation ability of melatonin (Olcese et al., 2009); enhanced brain antioxidant profiles and anti-inflammatory effects were also observed in the same melatonin-treated AD mice. Recently we reported clear evidence that chronic melatonin treatment to AD mice greatly enhanced mitochondrial function across multiple measures (Dragicevic et al., 2011). Although anti-aggregation effects of melatonin to break-up Aβ oligomers associated with brain mitochondria are one mechanism for this enhancement of mitochondrial function, we have recently identified a signaling pathway from melatonin receptors to mitochondria that plays an additional important role in the protection afforded by melatonin against Aβ-mediated mitochondrial dysfunction (Dragicevic et al., 2011). However, the molecular pathway that relays the signal from melatonin receptors to mitochondria has yet to be elucidated.

Epidemiological studies (Maia and de Mendonca, 2002) and studies with transgenic Alzheimer's mice have indicated that caffeine may be able to delay (Arendash et al., 2006) or even reverse cognitive dysfunction in AD (Arendash et al., 2009). Indeed, we have just reported that high plasma levels of caffeine are associated with no conversion of mild cognitive impairment (MCI) patients to AD (Cao et al., 2012). Our work in AD transgenic lines suggests the presence of multiple mechanisms of caffeine action to protect against AD – most notably, suppression of brain Aβ production and brain anti-inflammatory actions (Arendash et al., 2006, 2009). These caffeinergic mechanisms likely involve multiple effectors in the cell. For example, caffeine at physiological concentrations functions as an antagonist of adenosine receptors. In the brain, adenosine A_1 and A_{2A} receptors are present (Fredholm et al., 1999). Inhibition of adenosine A_{2A} receptors has been shown to be essential for the psychostimulant activity of caffeine (El Yacoubi et al., 2000) as well as protection of memory following Aβ administration in rodents (Cunha et al., 2008; Dall'Igna et al., 2007). At 10–100 fold higher concentrations than it blocks adenosine receptors, caffeine acts as a non-specific inhibitor of phosphodiesterases and the $GABA_A$ receptor (Fredholm et al., 1999). Microarray data of mice injected with caffeine showed that all three of these caffeine targets may play a role in the gene expression changes caused by high doses of caffeine, but at a lower (more physiologically relevant) dose of caffeine, adenosine receptors are the primary, but not the only target of inhibition (Yu et al., 2009).

We have shown that caffeine and melatonin can separately delay cognitive dysfunction in Alzheimer's mice (Arendash et al., 2006; Olcese et al., 2009). We reasoned that adding both together would be a potent therapeutic, because melatonin potently inhibits Aβ aggregation (Olcese et al., 2009) and increases mitochondrial function (Dragicevic et al., 2011), while caffeine decreases beta and gamma secretase activity to decrease Aβ production (Arendash et al., 2006) and increases Aβ clearance from the brain (Qosa et al., 2012). Since mitochondrial dysfunction may also play a role in loss of cognition in AD, the present study determined if one month oral treatment with caffeine, melatonin, or both in combination could reverse mitochondrial dysfunction in Alzheimer's mice.

2. Materials and methods

2.1. Mouse treatment protocol

All experimental protocols involving animals were approved by the University of South Florida Animal Care and Use Committee. Mice in this study were derived from the Florida Alzheimer's Disease Research Center mouse colony, wherein heterozygous mice carrying K670N and M671L mutations in the APP gene (referred to as the Swedish mutation or APP_{sw}) are routinely crossed with heterozygous mutant PS1 [Transgenic (Tg) line 6.2] mice to obtain APP_{sw}/PS1, APP_{sw}, PS1, and nontransgenic (NT) genotype offspring with a mixed C57/B6/SW/SJL background. In the present study, 11–12 month old APP_{sw} mice and littermate nontransgenic (NT) mice were divided into the following treatment groups: Tg + caffeine ($n = 3$), Tg + melatonin ($n = 4$), Tg + caffeine and melatonin ($n = 4$), Tg controls ($n = 4$), NT + caffeine ($n = 2$), NT + melatonin ($n = 2$), NT + caffeine and melatonin ($n = 2$), and NT controls ($n = 2$). Mice were placed on caffeine (120 mg/L), melatonin (500 mg/L) or both caffeine (120 mg/L) and melatonin (500 mg/L) in their drinking water or continued on normal water for one month. Since the mice studied drank an average of 5 ml water/day, their daily melatonin intake was calculated to be 2.5 mg and their average caffeine consumption was calculated to be 0.6 mg. This mode of administering melatonin or caffeine to rodents has been used previously (Resuehr and Olcese, 2005; Sharman et al., 2002a, 2002b).

2.2. Isolation of brain mitochondria from mice

Following the one month treatment period, animals were euthanatized using CO_2 asphyxiation and decapitated as previously described (Brown et al., 2004; Dragicevic et al., 2010). Brains were quickly removed and placed on ice. Brain regions of interest were carefully dissected following anatomical guidelines and placed in a glass Dounce homogenizer containing five times the volume of isolation buffer (215 mM mannitol, 75 mM sucrose, 0.1% BSA, 1 mM EGTA, 20 mM HEPES (Na^+), pH 7.2). Mitochondrial isolation was performed as in (Dragicevic et al., 2010). The final mitochondrial pellet was resuspended in isolation buffer without EGTA at a protein concentration of approximately 10 mg/ml and kept on ice for up to 2 h until the experiments were performed. Mitochondrial protein concentration was read using a BCA protein assay kit. For all mitochondrial analyses, the brain area of interest from 2 to 3 mice in a given group was combined for a single homogenate that was then assayed in triplicate. When there were 4 mice in a group, these 4 mice were separated into 2 groups of 2 mice per group. 3 assays were performed on the isolated mitochondrial homogenate from each group. Combining of individual tissues was necessary because the amount of tissue required for the entire suite of mitochondrial measures was more than any individual animal could provide for some of the brain areas evaluated.

2.3. Respiratory measurements

The respiratory function of isolated mitochondria was measured using a miniature Clark type oxygen electrode (Strathkelvin Instruments, MT200A chamber, Glasgow, UK). 100 μg (0.3 mg/ml final concentration) of mitochondria were suspended in a sealed, constantly stirred and thermostatically controlled chamber at 37 °C containing 0.35 ml of respiration buffer (125 mM KCl, 1 mM $MgCl_2$, 2 mM KH_2PO_4, 5 mM pyruvate, 2.5 mM malate, 500 μM EGTA, 20 mM HEPES, pH 7.0) at 37 °C. Maximum respiration was assessed by addition of 1 μM carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP) (uncoupler of oxidative phosphorylation). For cellular respiratory studies cells were grown on a 10 cm plate, treated with compounds for 48 h until confluency, then trypsinized, and suspended in 0.35 ml of phosphate buffered saline in a Clark type oxygen electrode as in (Frezza et al., 2007). The maximal respiratory rate was obtained following the addition of 10 μM FCCP.

2.4. Cell culture and treatment

N2a and N2a cells stably transfected with mutant APP_{sw} (N2a–APP_{sw}) (Thinakaran et al., 1996) were grown in standard high glucose (4.5 g/L) DMEM