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Effects of Cholinergic Lesions and Cholinesterase Inhibitors on Aromatase and Estrogen Receptor Expression in Different Regions of the Rat Brain

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Abstract—Cholinergic projections have been shown to interact with estrogens in ways that influence synaptic plasticity and cognitive performance. The mechanisms are not well understood. The goal of this study was to investigate whether cholinergic projections influence brain estrogen production by affecting aromatase (ARO), or influence estrogen signaling by affecting estrogen receptor expression. In the first experiment, ovariectomized rats received intraseptal injection of the selective immunotoxin 192IgG-saporin to destroy cholinergic inputs to the hippocampus. In the second experiment ovariectomized rats received daily intraperitoneal injections of the cholinesterase inhibitors donepezil or galantamine for 1 week. ARO activity and relative levels of ARO, ERα, ERß, and GPR30 mRNAs were quantified in the hippocampus, frontal cortex, amygdala and preoptic area. Results show that the cholinergic lesions effectively removed cholinergic inputs to the hippocampus, but had no significant effect on ARO or on relative levels of ER mRNAs. Likewise, injections of the cholinesterase inhibitors had no effect on ARO or ER expression in most regions of the brain. This suggests that effects of cholinergic inputs on synaptic plasticity and neuronal function are not mediated by effects on local estrogen production or ER expression. One exception was the amygdala where treating with galantamine was associated with a significant increase in ARO activity. The amygdala is a key structure involved in registering fear and anxiety. Hence this finding may be clinically relevant to elderly patients who are treated for memory impairment and who also struggle with fear and anxiety disorders.

Key words: local estrogen production, 192IgG-saporin, donepezil, galantamine, amygdala.

INTRODUCTION

The goal of this study was to explore potential effects of cholinergic manipulation on aromatase (ARO) activity and estrogen receptor (ERs) expression in different regions of the brain. Estrogens have been shown to have beneficial effects on learning, memory, and attention in multiple species including rats, mice, non-human primates, and in humans (Daniel et al., 1997; Bimonte and Denenberg, 1999; Luine et al., 2003; Gresack and Frick, 2006; Frye et al., 2007; Sherwin and Henry, 2008). Effects often are limited to females and are task specific. In rodents, estrogens (primarily estradiol (E2)) have been shown to enhance performance on a variety of spatial navigation tasks (Daniel et al., 1997; Fader et al., 1998; Gibbs and Johnson, 2008), and to enhance working memory (Bimonte and Denenberg, 1999; Daniel et al., 2006; Bohacek and Daniel, 2007), as well as novel object and object placement recognition (Luine et al., 2003; Frye et al., 2007; Fernandez et al., 2008). In humans beneficial effects have been observed on short-term and long-term verbal memory and logical reasoning (Sherwin, 1988; Krug et al., 2006). Estrogens also have been shown to enhance synapse formation, connectivity, and NMDA receptor expression in the hippocampus, with corresponding effects on synaptic transmission and long-term potentiation (McEwen et al., 2001; Jelks et al., 2007; Mendez et al., 2011). These effects are thought to underlie some of the effects of estrogens on cognitive performance.

We and others also have demonstrated that cholinergic projections from the medial septum (MS) to the hippocampus are significantly affected by estrogens and that these projections can play an essential role in enabling estrogen-mediated effects on cognitive performance. For example, ovariectomy reduces and E2 treatment increases choline acetyltransferase (ChAT)

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Abbreviations: ACh, acetylcholine; ARO, affecting aromatase; CamKII, calcium/calmodulin-dependent protein kinases; ChAT, choline acetyltransferase; ChEIs, cholinesterase inhibitors; CREB, cAMP response element-binding proteins; DMP, delayed-matching-to-position; ERs, estrogen receptors; IHC, immunohistochemistry; IR, immunoreactivity; MAPK, mitogen-activated protein kinases; MS, medial septum; NBM, magnocellularis; POA, preoptic area.

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mRNA in the MS and nucleus basalis magnocellularis (NBM), with corresponding effects on ChAT activity (Luine, 1985; Gibbs and Pfaff, 1992; Gibbs et al., 1994; Gibbs, 1996, 1997), high affinity choline uptake and acetylcholine (ACh) release in the frontal cortex and hippocampus (Gibbs et al., 1997; Gibbs, 2000; Gabor et al., 2003). E2 treatment has been shown to mitigate effects of scopolamine on T-maze alternation in rats (Fader et al., 1998), and likewise to mitigate effects of both scopolamine and mecamylamine on cognitive performance in post-menopausal women (Dumas et al., 2006). Notably, selective removal of cholinergic projections to the hippocampus prevents estrogen-mediated enhancement of a delayed-matching-to-position (DMP) spatial navigation task (Johnson et al., 2002; Gibbs and Johnson, 2007). Similar cholinergic lesions also have been shown to block estrogen-mediated increases in synaptic spines on CA1 neurons in the hippocampus (Lam and Leranth, 2003). More recent studies suggest that loss of ERs also contribute to loss of estrogen effects on cognitive function with age (Foster, 2012; Bean et al., 2014; Black et al., 2016), and that increasing ERα expression can enhance cognitive performance (Foster et al., 2008). We have shown that beneficial effects of E2 on DMP acquisition can be restored by treating older rats and rats with partial cholinergic lesions with selective cholinesterase inhibitors (ChEIs) (Gibbs et al., 2009, 2011a, b). Whether levels of ERs also were affected was not explored.

Collectively the findings demonstrate important interactions between basal forebrain cholinergic projections and estrogen effects on performance that impact brain aging and cognition. To date there has been little study of whether cholinergic projections significantly influence estrogen signaling, ER expression, or perhaps even local estrogen production, in the brain.

Estrogens are produced by the aromatization of androgens via the cytochrome P450 enzyme ARO. ARO is encoded by the CYP19A1 gene. In brain there are two isoforms of the gene. One is 430nt in length (ARO_L), and is associated with enzyme activity. The other is a truncated form 300nt in length (ARO_S), the function of which is unknown (Tabatadze et al., 2014). Recent studies demonstrate that local estrogen production in the adult brain can significantly influence brain structure and function (Garcia-Segura, 2008; Roselli et al., 2009; Stocco, 2012; Kato et al., 2013; Fester and Rune, 2015; Bender et al., 2017).

Estrogen effects are mediated by binding with specific estrogen receptors (ERs). Three receptors have been identified. ERα and ERβ are nuclear receptors that act as transcription factors for estrogen-regulated genes (Toran-Allerand, 2004). Studies show that these receptors also are located in specific cytoplasmic compartments where they can activate second messenger signaling pathways such as mitogen-activated protein kinases (MAPK), calcium/calmodulin-dependent protein kinases (CamKII), and cAMP response element-binding proteins (CREB) (McEwen, 2002; Manavathi and Kumar, 2006). A third receptor GPR30 is more recently identified and is a G protein-coupled receptor (Funakoshi et al., 2006; Moriarty et al., 2006; Brailoiu et al., 2007). It is located both intracellularly and on the plasma membrane and promotes rapid estrogen signaling in a variety of cell types. In the rat brain, GPR30 is present in many regions including the basal forebrain, cortex, hippocampus and hypothalamus (Brailoiu et al., 2007; Hazell et al., 2009). Studies from our lab have demonstrated that GPR30 is expressed by the majority of basal forebrain cholinergic neurons (Hammond et al., 2011), and that treatment with a GPR30 agonist increases ACh release in the hippocampus similar to the effects of E2 (Hammond et al., 2011; Gibbs et al., 2014).

Based on these findings, we proceeded to explore whether selective cholinergic lesions, as well as treatment with cholinesterase inhibitors, have effects on ARO expression, ARO activity, and ER expression in different regions of the brain.

EXPERIMENTAL PROCEDURES

Animals

Ninety-four ovariectomized (OVX, 270–350 g, 3 months old) Sprague–Dawley female rats were purchased from Harlan Sprague–Dawley Inc. Rats were individually housed for two weeks in our facility on a 12 h:12 h light/dark schedule with unrestricted access to food and water. All procedures were carried out in accordance with PHS policies and with the approval of the University of Pittsburgh's Institutional Animal Care and Use Committee.

In the first experiment, forty-six rats were used to test the effect of selective lesions of cholinergic neurons in the medial septum (MS) on aromatase (ARO) mRNA, activity and estrogen receptors (ERs) mRNA in the hippocampus and frontal cortex. Rats received intraseptal injections of 192IgG-Saporin (192IgG-SAP) or vehicle as described below. Two weeks later, rats were anesthetized with an overdose of ketamine (3 mg) and xylazine (0.6 mg). Brains were removed; hippocampal and frontal cortex tissues were collected and stored at −80 °C until use. Of the 46 rats, tissues from 20 rats (10/grp) were analyzed for relative levels of ARO and ER mRNAs using qRT-PCR methods described below. Tissues from the other 26 rats were analyzed for ARO activity (13 rats/grp) in microsomes using a recently validated and highly sensitive UPLC–MS/MS assay (Li et al., 2016). Immunohistochemical detection of ChAT-positive cells in the MS also was performed to confirm the loss of cholinergic neurons.

In the second experiment, a total of 48 rats were treated intraperitoneally with 3 mg/kg donepezil (Sigma–Aldrich, Inc.), 5 mg/kg galantamine (Sigma–Aldrich, Inc.) or saline (as control) injected once daily for 7 days. Donepezil and galantamine are cholinesterase inhibitors (ChEIs) approved for the treatment of memory decline associated with Alzheimer's disease. Following treatment rats were anesthetized and brain tissues were dissected as above. In addition to collecting hippocampus and frontal cortex, tissues from the

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