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Role of GPR30 in mediating estradiol effects on acetylcholine release in the hippocampus☆

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ABSTRACT

We have hypothesized that estradiol enhances basal forebrain cholinergic function and cognitive performance, at least in part, via activation of the novel estrogen receptor GPR30. Here we evaluated the effects of estradiol, G-1 (a selective GPR30 agonist), and tamoxifen (TAM; an ERα/ERβ antagonist that also acts as a GPR30 agonist), on acetylcholine (ACh) release in the hippocampus, as well as the ability to block the effects of 17β-estradiol (E) or TAM with the GPR30 antagonist G-15. Note that G-1 was included to evaluate the effects of selectively activating GPR30, whereas TAM was included to differentiate effects of E associated with activation of GPR30 vs. ERα or ERβ. The study was designed to test effects on potassium-stimulated release, as well as on ACh release stimulated by feeding. Effects of feeding were included because the tasks we used previously to demonstrate beneficial effects of E on cognitive performance were motivated by food reward, and we hypothesized that E may enhance performance by increasing ACh release in association with that reward. Ovariectomized rats were treated for 1 week, and ACh release was evaluated using in vivo microdialysis. In addition, rats were fed at the same time daily for several days and were fasted overnight prior to microdialysis. For each rat, ACh release was evaluated under basal conditions, in response to feeding, and in response to elevated potassium. Both feeding and elevated potassium increased ACh release in the hippocampus. In response to feeding, E, G-1, and TAM all significantly increased the percent change in release. The effects of E and TAM were blocked by G-15, and the effects of combining E + TAM did not differ significantly from the effects of E or TAM alone. In response to elevated potassium, E, and TAM significantly increased the percent change in ACh release. G-1 produced a slightly lesser effect. The effect of TAM was reduced by G-15, but the effect of E was not. These findings suggest that activation of GPR30 is both necessary and sufficient to account for the effects of E on ACh release associated with feeding. In contrast, activation of GPR30 appears to be sufficient, but may not be necessary for increased release associated with elevated potassium. The changes associated with feeding are consistent with the effects of E, G-1 and G-15 on acquisition of a spatial learning task previously described. These data confirm and extend previous reports, and support a hypothesis wherein E treatment can improve learning on specific tasks by activating GPR30 and enhancing ACh release in association with food reward.

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Introduction

Studies show that, in rodents, ovariectomy impairs and estradiol enhances performance on a variety of learning, memory, and attention tasks (Bimonte-Nelson et al., 2010; Daniel and Bohacek, 2010; Gibbs, 2010). We hypothesize that these effects are mediated, at least in part, by effects on cholinergic inputs to the hippocampus and cortex (Gibbs, 2010). This is supported by studies showing that 17β-estradiol (E) enhances basal forebrain cholinergic function as evidenced by increases in choline acetyltransferase (ChAT) (Gibbs, 1996, 1997, 2000; Gibbs and Pfaff, 1992; Gibbs et al., 1994; Luine, 1985; Singh et al., 1994), high affinity choline uptake in the hippocampus and frontal cortex (Gibbs, 2000; O'Malley et al., 1987), and increases in potassium-stimulated acetylcholine (ACh) release (Gabor et al., 2003; Gibbs et al., 1997b). In addition, selective destruction of cholinergic neurons in the medial septum blocked the effects of estradiol on learning in a delayed matching-to-position (DMP) T-maze task (Gibbs, 2007), as well as on synaptic spine density in the hippocampus (Lam and Leranth, 2003). Intrahippocampal infusion of muscarinic cholinergic receptor inhibitors likewise blocked E effects on acquisition of an 8-arm radial maze task (Daniel et al., 2005). Conversely, treating with cholinesterase inhibitors partially restored estradiol effects on learning in aged rats (Gibbs et al., 2009, 2011a), and in young rats with partial cholinergic lesions (Gibbs et al., 2011b). These findings indicate that effects of E on cholinergic function are an important mediator of effects on performance, at least within specific cognitive domains.

The mechanisms by which E mediates the effects on basal forebrain cholinergic neurons are not well understood. A subset of the cells (~10–

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30%) contains ERα (not ERβ) (Miettinen et al., 2002; Shughrue et al., 2000) and there is some evidence for direct effects mediated via this receptor (Pongrac et al., 2004; Szego et al., 2006). Recently, however, we found that the majority of the cholinergic neurons contain GPR30, a novel G protein-coupled estrogen receptor (Hammond et al., 2011). Hence current studies are focusing on the role of GPR30 in mediating estrogen effects on cholinergic function and cognitive performance. To that end we have shown that G-1, a selective GPR30 agonist, increases potassium-stimulated ACh release in the hippocampus and enhances acquisition of the DMP task similar to E (Hammond et al., 2009, 2011). Furthermore G-15, a selective GPR30 antagonist, impaired the performance of gonadally intact rats comparable to the effect of ovariectomy, and blocked the ability of E to enhance performance in ovariectomized rats (Hammond et al., 2012). This suggests that GPR30 plays an essential role in mediating the effects of E on acquisition of this task.

The current report extends our recent findings by further evaluating the role of GPR30 in mediating the effects of E on ACh release. Here we evaluated not only potassium-stimulated release, but also release associated with the presentation of food following an overnight fast. This modeled, in part, the food reward associated with acquisition of the DMP task. In vivo microdialysis was used to compare the effects of E, G-1 and tamoxifen (an ERα antagonist/GPR30 agonist) on ACh release. We also tested the ability to block effects of E and tamoxifen (TAM) with G-15. Results show that E, G-1, and TAM all increased ACh release, both potassium-stimulated release as well as release associated with feeding. The effects of E and TAM on release associated with feeding were completely blocked by G-15. In contrast, the effects of E on potassium-stimulated release were not blocked by G-15. This suggests that activation of GPR30 is both necessary and sufficient to mediate the effect of E on ACh release associated with food reward, and suggests that this effect may underlie the effects of E, G-1 and G-15 on DMP acquisition previously described.

Methods

Animals

A total of 52 young adult female Sprague–Dawley rats (~3 months old) were purchased from Hilltop Laboratories. All rats were ovariectomized (Ovx) by the supplier prior to delivery. Rats were individually housed on a 12-hour day/night cycle (7 am to 7 pm) with food and water freely available. Rats were housed for at least 2 weeks prior to treatments. All procedures were carried out in accordance with PHS policies on the use of animals in research, and with the approval of the University of Pittsburgh's Institutional Animal Care and Use Committee.

Drug treatments

E was delivered by silastic capsule (6 mm length, 1.98 mm I.D., 3.18 mm O.D.) packed with 3 mm of powdered 17β-estradiol (Sigma-Aldrich, Inc.) implanted s.c. in the dorsal neck region. G-1, and G-15 were dissolved first in DMSO and then in 20% β-hydroxypropyl cyclodextrin (Aldrich, Inc.) and then administered by miniosmotic pump (Alzet model 2002; Durect, Corp.) implanted s.c. in the dorsal neck region. Tamoxifen (TAM; Tocris, Inc.) was dissolved in saline and administered daily by injection s.c. Controls received pumps containing vehicle as well as daily injections of saline.

Treatments were as follows:

$$\text{Controls}(n=9);\ \ \text{E}(n=9);\ \ \text{G}-1\text{–}5\ \mu\text{g/day}(n=5);$$
$$\text{Tam–2mg/kg/day}(n=5);\ \ \text{E}+\text{TAM}(n=5);\ \text{G}-15\text{–}40\mu\text{g/day}(n=6);$$
$$\text{E}+\text{G}-15(n=8);\ \ \text{TAM}+\text{G}-15(n=5).$$

Doses of E and G-1 were selected based on previous studies showing significant effects on DMP acquisition (Gibbs, 2007; Hammond et al., 2009) or effects on potassium-stimulated release (Gabor et al., 2003; Hammond et al., 2011). In addition, the dose of E was estimated to produce circulating levels of E in the normal physiological range (~50 pg/mL). An identical dose of G-1 was used based on similarities to E in molecular weight and structure. At the time of pump implantation rats also received a microdialysis guide cannula (CMA 12 Elite Guides, CMA Microdialysis, Inc.) lowered into the right hippocampus (−3.4 mm bregma, 1.18 mm lateral, −3.4 mm ventral) and fixed to the skull using dental cement as previously described (Gibbs et al., 2004). Microdialysis was performed 7 days later.

Feeding

Rats were fed at the same time in the morning of each day for several days prior to microdialysis. This was done so that rats would learn to anticipate food at a particular time each day. This is similar to the restricted feeding paradigm that was used when rats were trained to perform the DMP task described in prior reports.

In vivo microdialysis

Rats were fasted overnight prior to microdialysis. Concentric, 3 mm microdialysis probes were used (CMA 12 Elite Probes, CMA Microdialysis, Inc.). On each day of microdialysis, probes were first dialyzed for 30 min against a solution of ACSF containing 0.1 pmol ACh/20 μL. This sample was used to calculate and correct for probe efficiency. The probe was then rinsed and the infusate changed to ACSF containing 0.2 μM neostigmine. The neostigmine was added based on previous studies showing that modest inhibition of acetylcholinesterase activity is necessary in order to reliably detect basal levels of acetylcholine in the extracellular fluid (Gabor et al., 2003; Gibbs et al., 1997a; Rhodes et al., 1997). The probe was then inserted through the cannula into the hippocampus and the rat was placed into a large plastic container for the duration of the experiment. The probe was perfused at a rate of 1 μL/min and dialysate was collected continuously. Samples were collected and frozen every 30 min. After waiting 90 min for basal ACh release to stabilize, two samples were collected over 1 h and averaged to represent basal release. Rats were then given food pellets every 5 min for 15 min and dialysate was collected for 30 min. This sample was used to assess ACh release in response to feeding. Next, probes were perfused with ACSF containing elevated levels of potassium (60 mM) and samples were collected after 30 and 60 min to measure potassium-stimulated release. Following microdialysis rats were given an overdose of ketamine (40 mg/kg) and xylazine (28 mg/kg) injected i.p. and euthanized by decapitation.

Quantification of 17β-estradiol

Trunk blood was collected for the determination of serum estradiol levels using a sensitive LC–MS–MS method recently described (Hammond et al., 2012). Briefly, Samples were spiked with internal standard (2,4,16,16,17-d3-17β-estradiol) and then extracted with n-butyl chloride. After centrifugation and evaporation, the residue was derivatized in 0.1 mL buffered dansyl chloride (pH 10.5). E2 was eluted from a Waters Acquity UPLC BEH C18, 1.7 μm, 2.1 × 150 mm reversed-phase column, with an acetonitrile:water (0.1% formic acid) gradient. Detection and quantification were achieved in the positive mode. Transitions used for analysis were 506 → 171 for E2, and 511 → 171 for the deuterated internal standard. Area under the peak was quantified and used to determine absolute levels of E2/mL of sample by comparison with a series of standards. The limit of detectability for this assay was 2.5 pg/mL.

Quantification of ACh

Levels of ACh in the dialysates were measured by HPLC with electrochemical detection as previously described (Hammond et al., 2011).

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