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Estradiol and ERβ agonists enhance recognition memory, and DPN, an ERβ agonist, alters brain monoamines

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ABSTRACT

Effects of estradiol benzoate (EB), ERα-selective agonist, propyl pyrazole triol (PPT) and ERβ-selective agonists, diarylpropionitrile (DPN) and Compound 19 (C-19) on memory were investigated in OVX rats using object recognition (OR) and placement (OP) memory tasks. Treatments were acute (behavior 4 h later) or sub chronic (daily injections for 2 days with behavior 48 h later). Objects were explored in sample trials (T1), and discrimination between sample (old) and new object/location in recognition trials (T2) was examined after 2–4 h inter-trial delays. Subjects treated sub chronically with EB, DPN, and C-19, but not PPT, discriminated between old and new objects and objects in old and new locations, suggesting that, at these doses and duration of treatments, estrogenic interactions with ERβ contribute to enhancements in recognition memory. Acute injections of DPN, but not PPT, immediately after T1, also enhanced discrimination for both tasks (C19 was not investigated). Effects of EB, DPN and PPT on anxiety and locomotion, measured on elevated plus maze and open field, did not appear to account for the mnemonic enhancements. Monoamines and metabolites were measured following DPN treatment in subjects that did not receive behavioral testing. DPN was associated with alterations in monoamines in several brain areas: indexed by the metabolite, 3-methoxy-4-hydroxyphenylglycol (MHPG), or the MHPG/norepinephrine (NE) ratio, NE activity was increased by 60–130% in the prefrontal cortex (PFC) and ventral hippocampus, and NE activity was decreased by 40–80% in the v. diagonal bands and CA1. Levels of the dopamine (DA) metabolite, homovanillic acid (HVA), increased 100% in the PFC and decreased by 50% in the dentate gyrus following DPN treatment. The metabolite of serotonin, 5-hydroxyindole acetic acid (5-HIAA), was increased in the PFC and CA3, by approximately 20%. No monoaminergic changes were noted in striatum or medial septum. Results suggest that ERβ mediates sub chronic and acute effects of estrogens on recognition memory and that memory enhancements by DPN may occur, in part, through alterations in monoaminergic containing systems primarily in PFC and hippocampus.

1. Introduction

That estradiol modulates neural functions like mood, affect, anxiety, fear and vulnerability to addictive drugs in addition to its well documented role in reproduction is now well accepted (Watson, Alyea, Cunningham, & Jeng, 2010). Moreover, estradiol also exerts influence over higher order cognitive function, predominantly enhancing learning and memory (Luine, 2008). However, regulation of cognition by estrogens and other hormones is complex with efficacy dependent on dose, duration of treatment and nature of the cognitive demand (Frick, 2009; Luine, 2008). For example, high levels of estradiol have been shown to inhibit performance of some memory tasks and may even impair performance once subjects have learned how to solve the task (Dohanich, 2002), and recent studies indicate that estrogens may influence strategies used for solving memory tasks which may lead to poorer performance in females as compared to males (Davis, Jacobson, Aliakbari, & Mizumori, 2005; Korol, 2004). However, a large body of evidence in both young and aged rodent subjects indicates that estradiol promotes memory (Daniel, 2006; Frick, 2009; Luine, 2008). For example, enhanced memory has been shown when estrogens are given by chronic (i.e. days to weeks, Davis et al., 2005; Gibbs, Gabor, Cox, & Johnson, 2004; Luine, Richards, Wu, & Beck, 1998), sub chronic (i.e. a few days, Daniel & Dohanich, 2001; Frye & Rhodes, 2002; Leuner, Mendolia-Loffredo, & Shors, 2004; Sandstrom & Williams, 2004), or acute (i.e. min to h, Inagaki,

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Gautreaux, & Luine, 2010; Luine, Jacome, & MacLusky, 2003; Packard & Teather, 1997; Rhodes & Frye, 2006) treatment regimens to ovariectomized (OVX) rats in a variety of tasks, such as the delayed matching-to-position (DMP) T-maze, Morris water maze, eight-arm radial maze, object recognition, trace eyeblink conditioning and inhibitory avoidance tasks.

Effects of estrogens are mediated through receptors (ERs), and for almost 50 years, it was thought that this receptor was a single ligand-dependent transcription factor acting in the nucleus to enhance nuclear transcription (genomic effects). A second estrogen receptor was identified approximately 15 years ago (Kuiper, Enmark, Pelto-Huikko, Nilsson, & Gustafsson, 1996), and receptors are currently designated as ERα and ERβ. In addition, more recent results support nongenomic steroid actions initiated at the level of the cell membrane through ERs spanning the plasma membrane that mediate effects on pathways linked to G-protein and tyrosine kinase pathways (Roepke, Ronnekleiv, & Kelly, in press). ERs may also act directly at nuclear sites (CREB and AP1) to regulate transcription (Watson et al., 2010). Thus, estrogen effects are no longer limited to those that are delayed in onset and long lasting (chronic, genomic), but may also be rapid and short-lived (acute, membrane dependent).

In relation to memory, evidence suggests that ERβ, rather than ERα, contributes. Experiments in mice with knockouts of ERα (ERKO) or ERβ (BERKO) indicate that ERβ mediates learning and memory function by estradiol (Liu, Day, Muñiz, & Bitran, 2008; Rissman, Heck, Leonard, Shupnik, & Gustafsson, 2002; Walf, Koonce, & Frye, 2008). These results are supported by other experiments where estradiol and ER-specific agonists were given to OVX rodents. For example, estradiol and the ERβ agonist, WAY-200070, but not the ERα agonist, PPT (Harris, Katzenellenbogen, & Katzenellenbogen, 2002), given for 2 days, enhanced spatial memory on the radial arm maze (Liu et al., 2008). A similar pattern was found on the Morris water maze where estradiol and DPN, another ERβ agonist (Minutolo, Macchia, Katzenellenbogen, & Katzenellenbogen, 2009), but not PPT, given acutely, enhanced performance in OVX rats (Rhodes & Frye, 2006). However, other results do not support the view that estradiol effects on memory are mediated solely by ERβ. Using the spatial memory task, object placement, and acute treatments, Frye, Duffy, and Walf (2007) reported that estradiol and PPT, but not DPN, enhanced memory, and in the non-spatial memory test, object recognition, acute estradiol, PPT and DPN enhanced performance (Walf, Rhodes, & Frye, 2006). Finally, a recent study gave chronic treatments to OVX rats and tested acquisition and memory in a delayed matching-to-position (DMP) T-maze task (Hammond, Mauk, Ninaci, Nelson, & Gibbs, 2009). Estradiol, PPT and DPN all enhanced acquisition/learning as compared to OVX females; however, no treatment altered memory in tests with long inter-trial delays following the acquisition trials. Thus, it is currently unclear which ERs contribute to cognitive function or whether the two receptors may differentially regulate learning as compared to memory function or regulate different types of memory. Further confounding cognitive analyses is the observation that estradiol and ER agonists can influence overall activity and anxiety which might indirectly alter mnemonic function (Bowman, Ferguson, & Luine, 2002; Díaz-Véliz, Alarcón, Espinoza, Dussaubat, & Mora, 1997; Lund, Rovis, Chung, & Handa, 2005; Tomihara et al., 2009).

In this study, we examined estrogenic influences on cognitive function using the non-spatial memory task, objection recognition (OR), and the spatial memory task, object placement (OP). These tasks are advantageous since they provide an assessment of working memory with little learning component (Ennaceur, Neave, & Aggleton, 1997). Effects of estradiol were first evaluated, and then the ERα agonist, PPT, and the ERβ agonists DPN and compound 19 (Wilkening et al., 2006) were tested. Both sub chronic (days) and acute (h) treatments were assessed in an attempt to determine which ER receptors may be responsible for enhancements in memory. In addition, the effects of estradiol and some of the ER ligands were evaluated in the open field and elevated plus maze to assess possible non-mnemonic contributions to performance in memory tasks. Finally, effects of DPN on monoamine and metabolite levels in specific brain areas were measured to determine whether agonist-dependent changes in monoaminergic function are present in areas contributing to cognitive function because monoamines have been previously shown to contribute to cognition (Brozoski, Brown, Rosvold, & Goldman, 1979; Ramos & Arnsten, 2006).

2. Materials and methods

2.1. Subjects

Two-month old, female Sprague Dawley rats were OVX by the vendor (Harlan Sprague Dawley, Inc., Indianapolis, IN) and delivered to Hunter College where they were double-housed in plastic cages and kept on a 12/12 h light/dark cycle (lights on at 07:00am) with access to food and water *ad libitum*. A diet very low in phytoestrogens (Chow 2016, 16% protein rodent diet, Harlan Teklad Global Diets, Madison, WI) was provided because OVX rats on regular rat chow show enhanced spatial memory and increased dendritic spine density in hippocampal and prefrontal cortex (PFC) pyramidal neurons (Luine, Attalla, Mohan, Costa, & Frankfurt, 2006). All experiments conformed to the guidelines of the NIH Guide for Care and Use of Animals and were approved by the Institutional Animal Care and Use Committee of Hunter College.

2.2. Sub chronic hormonal treatment

Cohorts of 16–18 OVX rats each were used in the behavioral testing (EB, PPT and DPN, C19), and an additional, separate cohort of 16 OVX rats was used for monoamine measurements. After acclimation (discussed below), half of the subjects (n = 8–9/group) received a single daily sc injection of either EB (50 μg/kg), PPT (3 and 5 mg/kg), DPN (3 mg/kg), or C-19 (3 and 5 mg/kg), or vehicle (sesame oil or propylene glycol; 200–300 μl) for 2 days and were tested 2 days after the last injection. EB, rather than estradiol, was utilized because it provides more sustained and longer elevations in circulating estradiol (Scharfman et al., 2007). We have previously shown that this EB dose increases CA1 dendritic spine synapse density (MacLusky, Luine, Hajszan, & Leranth, 2005). PPT and DPN, at the doses given or at similar doses, exert estrogenic effects. For example, PPT and E_2, but not DPN, increase uterine weight (Harris et al., 2002; Le Saux & Di Paolo, 2005; Lubbers et al., 2006; Lund et al., 2005), whereas DPN and E_2, but not PPT, increase specific binding to dopamine transporters (DAT) in the rat striatum (Le Saux & Di Paolo, 2006). The doses of C-19 were chosen based on the compound's affinity and specificity for ERβ (Opas et al., 2009; Wilkening et al., 2006) since information on its effects in the CNS are not available. Two additional cohorts of subjects received a single daily sc injection of EB, drugs or vehicle for 2 days and were tested on the EPM 24 h after the second injection day and then tested for OR or OP on the following day to assess drug effects on anxiety and to confirm positive treatment effects on memory. Another cohort received acute DPN and was tested on the EPM 4 h later.

PPT and DPN were purchased from Tocris Bioscience (Ellisville, MO). EB was purchased from SIGMA (St. Louis, MO), and C-19 was a gift from Merck Research Laboratories. EB and PPT were dissolved in ethanol and then diluted in sesame oil. DPN and C-19 were dissolved in propylene glycol because they are not soluble in ethanol/sesame oil. Control subjects received the appropriate vehicle,