Gonadectomy and Hormone Replacement Affects In Vivo Basal Extracellular Dopamine Levels in the Prefrontal Cortex but Not Motor Cortex of Adult Male Rats

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Gonadectomy in adult male rats is known to impair performance on dopamine (DA)-dependent prefrontal cortical tasks and selectively dysregulate end points in the mesoprefrontal DA system including axon density. In this study, in vivo microdialysis and high-pressure liquid chromatography were used to determine whether short (4-day) and/or long-term (28 day) gonadectomy and hormone replacement might also influence the more functionally relevant metric of basal extracellular DA level/tone. Assessments in medial prefrontal cortex revealed that DA levels were significantly lower than control in 4-day gonadectomized rats and similar to control in 28-day gonadectomized animals supplemented with both testosterone and estradiol. Among the long-term treatment groups, DA levels were significantly higher than control in gonadectomized rats and gonadectomized rats given estradiol but were similar to control in rats given testosterone. In contrast, extracellular DA levels measured in motor cortex were unaffected by long- or short-term gonadectomy. The effects of gonadectomy and hormone replacement on prefrontal cortical DA levels observed here parallel previously identified effects on prefrontal DA axon density and could represent hormone actions relevant to the modulation of DA-dependent prefrontal cortical function and perhaps its dysfunction in disorders such as schizophrenia, attention deficit hyperactivity disorder, and autism where males are disproportionately affected relative to females.

Keywords: androgen, estrogen, HPLC, microdialysis, schizophrenia

Introduction

The prefrontal cortices (PFC) are important mediators of highest order information processing and controllers of functions such as working memory and behavioral flexibility (Goldman-Rakic et al. 1990; Dalley et al. 2004). These complex functions are exquisitely sensitive to local dopamine (DA) signaling, and information from gene polymorphisms and disease processes in humans (Davis et al. 1991; Goldberg et al. 2003) and from studies in experimental animal models (Tassin et al. 1978; Kessler and Markowitsch 1981; Kalsbeek et al. 1989; Murphy et al. 1996; Verma and Moghaddam 1996; Zahrt et al. 1997; Morrow et al. 2000) sum to suggest that both increased and decreased DA tone can adversely affect DA-dependent PFC function. In rats, for example, chemical lesions of PFC DA afferents (Tassin et al. 1978; Kessler and Markowitsch 1981; Kalsbeek et al. 1989; Sam et al. 1989), local administration of DA D1 receptor agonists and antagonists (Zahrt et al. 1997; Winter et al. 2009) and N-methyl D-aspartate receptor antagonists (Seamans et al. 1995; Verma and Moghaddam 1996), as well as beta-carboline- and stress-induced increases in DA turnover (Murphy et al. 1996; Morrow et al. 2000; Moghaddam and Jackson 2004) all adversely affect performance in frontal lobe-dependent tasks including open-field testing, delayed alternation paradigms, and novel object recognition. Given the more recent evidence showing that performance on these same DA-dependent PFC tasks is also sensitive to gonadal hormones (Einon 1980; van Haaren et al. 1990; Janowsky et al. 2000; Lacreuse 2006; Luine 2008), the question arises as to whether these behavioral influences of gonadal steroids are related to their modulation of PFC DA signaling. To begin to address this, methods of in vivo microdialysis were paired with gonadectomy and hormone replacement in adult male rats to determine whether these hormone manipulations affect the functionally pivotal metric of basal PFC DA tone.

Although assessments of sex differences (Dawson et al. 1975; Einon 1980; van Hest et al. 1988; Overman 2004; Shanks et al. 2004; Stanton et al. 2009) and of ovarian hormone influence in females (Rapp et al. 2003; Wide et al. 2004; Sinopoli et al. 2006; van Wingen et al. 2007; Frye and Walf 2008; Hatta and Nagaya 2009) are more numerous, a growing number of studies demonstrate significant hormone sensitivity of PFC function in males. In humans, for example, positive correlations have been identified between circulating testosterone level and PFC function in healthy subjects while negative correlations have been observed between testosterone titers and the severity of cognitive deficits in aging (Janowsky 2006), androgen deprivation therapies (Nelson et al. 2007), and in disorders such as schizophrenia (Shirayama et al. 2002; Goyal et al. 2004; Taherianfard and Shariaty 2004; Akhondzadeh et al. 2006; Ko et al. 2007). In adult male rats, gonadectomy (GDX) and/or hormone replacement has also been shown to significantly impair acquisition (Ceccharelli et al. 2001; Kritzer et al. 2001, 2007; Daniel et al. 2003) and/or negatively impact performance (Adler et al. 1999; Sandstrom et al. 2006; Aubele et al. 2008; Gibbs and Johnson 2008) in open-field testing, maze and operant versions of the spatial delayed alternation task, extradimensional set-shifting/behavioral flexibility, and novel object recognition—which are all tasks that are well known to be sensitive to PFC lesions (Bubser and Schmidt 1990; Barker et al. 2007; Naneix et al. 2009; Tait et al. 2009) and to selective mesoprefrontal DA perturbations (Tassin et al. 1978; Kessler and Markowitsch 1981; Kalsbeek et al. 1989; Stam et al. 1989; Murphy et al. 1996; Verma and Moghaddam 1996; Zahrt et al. 1997; Morrow et al. 2000). The possibility that the effects of GDX on these tasks are linked to hormone actions on the mesoprefrontal DA system is suggested first by findings that GDX selectively affects both PFC function and PFC DA axon density in an androgen-sensitive, estrogen-insensitive manner (Kritzer et al. 1999, 2001; Kritzer 2000) and further in studies showing that for several tasks, these functional and axon density effects are significantly correlated to one another (Kritzer et al. 2007). However, because the end point of innervation density may not reflect a functionally relevant...
index of PFC DA signaling, the studies presented here used in vivo microdialysis to ask whether GDX and/or hormone replacement might also affect basal PFC DA tone. Thus, using the same adult male rat models used in previous behavioral studies (Kritzer et al. 2001, 2007; Aubele et al. 2008) and/or anatomical analyses of cortical DA axon density (Kritzer et al. 1999; Kritzer 2000), basal extracellular DA level was measured in the medial prefrontal cortex (mPFC) of rats that had been sham operated, gonadectomized or gonadectomized, and supplemented with testosterone propionate (TP) or estradiol (E) for 4 or 28 days—time points where GDX is known to significantly decrease and increase PFC DA axon density, respectively. For comparison, extracellular DA level was also measured in the nearby and DA-enriched motor cortical fields of separate cohorts of 4- and 28-day GDX and control animals.

Materials and Methods

Animal Subjects

A total of 64 adult male Sprague-Dawley rats (Taconic Farms) were used. Animals were housed in pairs of like treatment under a 12:12 h light:dark cycle with food and water available ad libitum. Twenty animals were sham operated (CTRL); 9 were used in experiments 4 days after surgery, and the remainder were used after 28 days. The remaining 44 animals were gonadectomized (GDX); 9 subjects made up the 4-day GDX cohort and 12 were used in the 28-day GDX group. Thirteen GDX rats were supplemented with TP (GDX-TP): 5 were used for the 4-day cohort and 8 were used in the 28-day GDX-TP group. Ten GDX animals were supplemented with 17β-estradiol (GDX-E): 5 animals were used in the 4-day GDX-E cohort and 5 were used in the 28-day GDX-E group. All procedures involving animals were approved by the Institutional Animal Care and Use Committee at Stony Brook University and were designed to minimize their use and discomfort.

Surgery

All surgeries were carried out under aseptic conditions and used intraperitoneal injections of ketamine (0.9 mg/kg) and xylazine (0.5 mg/kg) as anesthesia. Rats were monitored during recovery from surgery and given 0.03 mg/kg buprenorphine to manage any post-operative discomfort.

1) Sham surgery or gonadectomy was performed 4 or 28 days before microdialysis. For both procedures, the sac of the scrotum and the underlying layers of tunica were incised. For GDX, the vas deferens was also bilaterally ligated and the testes removed. Incisions were closed using sterile wound clips that were removed 2 weeks later for the 28-day survival animals.

2) Craniotomy was performed for placement of microdialysis probe guide cannulae on the day prior to the microdialysis experiment. For this procedure, anesthetized rats were placed in a stereotaxic frame (Kopf Instruments), an incision was made to expose the skull, and burr holes were drilled for inserting and anchoring guide cannulae (CMA Microdialysis) to the skull. Using coordinates adapted from the atlas of Paxinos and Watson (1998), cannulae were aimed toward either the left pregenual mPFC or left primary motor cortex located 3.2 mm anterior to Bregma and were secured with a combination of shallow screws and dental cement.

Hormone Supplements

For the hormone-supplemented groups, pellets that released either 3-4 ng of TP (GDX-TP rats) or 25 pg of E per milliliter of blood per day (GDX-E rats; Innovative Research of America) were inserted within the tunica at the time of GDX. These pellets and doses have been used in previous investigations in this laboratory and others and have been shown to yield circulating level of gonadal hormones that fall within the physiological range (Collins et al. 1992; Adler et al. 1999; Kritzer 2000).

In Vivo Microdialysis

On the morning after craniotomy, awake, freely moving animals were placed in Ratum clear rodent bowls (BioAnalytical Systems) and allowed to acclimate for 30 min before microdialysis probes (100 000 Dalton cutoff, 3 mm polyethersulfone-exposed membrane tip; CMA Microdialysis) were slowly lowered through guide cannulae into place. For the next 2 h, the probes were perfused with artificial cerebrospinal fluid (145 mM NaCl, 2.8 mM KCl, 1.2 mM MgCl2, 0.25 mM ascorbic acid, 5.4 mM glucose, 1.2 mM CaCl2 in 1 L H2O, pH 6.8) at a rate of 2 μL/min, during which time subjects fell asleep. Afterward, dialysates were collected every 20 min from PFC and/or motor cortex. This phase of the experiment was between 9 AM and 6 PM that corresponded to the rats’ subjective night. At least 3 samples were collected from each animal at times when they were observed to be sleeping; for the very few cases where a given 20-min sample was collected while an animal was transiently awake, that sample as well as the one collected over the next 20 min were excluded from the analysis due to the increases in cortical DA levels that are known to occur with arousal and movement. Dialysate samples (5 μL) were directly injected into an high performance liquid chromatography (HPLC) system (PM 92-E pump; BAS) via an on-line autosampler (Pollen-8; BAS). Sample analyses utilized a microcolumn (Unijet, 1.0 mm inner diameter, 150 mm length, 5 μm octyldicyl silane particles; BAS) and a BioAnalytical Systems LC-Epsilon detector (BAS); the E

Epsilon detector (BAS) app was + 0.65 V versus the Ag/AgCl reference electrode and the mobile phase consisted of 14.5 mM NaH2PO4, 30 mM sodium citrate, 10 mM diethylamine HCl, 2.2 mM 1-octanesulfonic acid, 0.027 mM EDTA, 7.2% acetonitrile (v/v), and 1% tetrahydrofuran (v/v), pH 3.4. Probe efficiency was measured at 12-18% for all studies, and an overall detection limit of 10 fmol was achieved. All chemicals used were purchased from Sigma-Aldrich Chemical Co.

Euthanasia and Histology

At the conclusion of the microdialysis study, animals were euthanized via rapid decapitation and their brains were removed and immersed in a 30% sucrose, 10% buffered formaldehyde solution for fixation, and cryoprotection. The androgen-sensitive medial ventral and lateral bulboponsunglous muscles (BSM) were also dissected out and weighed. After the brains sank in the cryosection solution, the frontal cortex region of each subject was blocked, rapidly frozen, and serially sectioned in the coronal plane on a freezing microtome at a thickness of 40 μm. For each animal subject, a 1 in 4 series of sections taken from mid-olfactory bulb through the genu of the corpus callosum was collected, slide mounted, Nissl stained, and examined to verify the cytoarchitectonic location of the microdialysis probe. In order for data from a given animal to be included in the analyses, the following criteria had to be met: for the PFC, probe tips had to be placed in the left pregenual mPFC, extend dorsoventrally through prelimbic and infralimbic areas but not beyond to dorsopeduncular cortex, and be centered over middle and deep cortical layers (see Fig. 1); with this placement, the 2-mm probe tip length covered about 1 mm in each of these 2 medial prefrontal fields. For studies in the motor cortex, probes had to be placed on the lateral cortical surface within the left premotor (M2) and/or primary motor (M1) field and not extend beyond into white matter, claustrum, or the dorsal or ventral anterior insular cortices (AID, AIV) (see Fig. 2).

Data Analysis

Dopamine peak identity was confirmed and quantified (ng/10 mL) in relation to standard peaks of known concentrations (1, 2, 5, and 10 ng/10 mL) run through the HPLC system on the same day as the animal data were collected; uncorrected peak values were measured automatically by ChromGraph software (BAS) as peak heights and data were collected; uncorrected peak values were measured automatically by ChromGraph software (BAS) as peak heights and relation to standard peaks of known concentrations (1, 2, 5, and 10 ng/10 mL) run through the HPLC system on the same day as the animal data were collected; uncorrected peak values were measured automatically by ChromGraph software (BAS) as peak heights and
used the Fisher's protected least significant difference, and in all cases, P < 0.05 was accepted as significant. BSM weights were also assessed using the same descriptive and comparative statistical methods, and regression analyses in which the BSM weights were used as independent variables and the individual animals' extracellular PFC DA (above) were used as dependent variables were also carried out.

Results

Effectiveness of Hormone Treatments
The weights of the androgen-sensitive BSMs showed expected group differences among both the 4- and 28-day treatment groups. Specifically, in both cases, the mean muscle weights of the CTRL and GDX-TP rats were higher than those of the GDX and GDX-E cohorts. However, as expected as well, the between-group differences in muscle weights were more pronounced for the 28- compared with the 4-day treatment groups (see Fig. 3). Nonetheless, separate ANOVA that compared individual animal BSM weights revealed significant main effects of hormone treatment for both the longer and shorter treatment groups (4 day: $F_{3,15} = 10.471$, $P = 0.0006$; 28 day: $F_{5,23} = 339.432$, $P < 0.0001$). Allowed post hoc tests further confirmed that for both sets of animals, the BSM weights of the control and GDX-TP cohorts were similar to and not significantly different from one another, the weights of the GDX and GDX-E rats were similar to and not significantly different from each other and that the BSM weights of the 4- and 28-day GDX and GDX-E cohorts were both significantly different from those of the corresponding control and GDX-TP rats (Fig. 3).

Extracellular Prefrontal Cortical Dopamine Level

4-Day Treatment Groups
Extracellular levels of DA measured in the left pregenual mPFC of the 4-day sham-operated (CTRL) rats were similar to values previously reported in in vivo microdialysis studies of DA in the mPFC of adult male rats (Moghaddam and Jackson 2004; Stefani and Moghaddam 2005; Del Arco et al. 2007; van der Meulen et al. 2007; Balla et al. 2009). Thus, values in the CTRL group ranged from 0.10 to 0.23 fmol/μL and had an average of 0.15 ± 0.02 fmol/μL. For the GDX cohort, however, basal extracellular PFC DA level was roughly 40% lower (range of 0.08-0.11 fmol/μL, mean of 0.09 ± 0.004 fmol/μL) while PFC DA level in both hormone-supplemented groups (GDX-TP and GDX-E rats) was similar to controls (GDX-TP: range of 0.11-0.21 fmol/μL, mean 0.16 ± 0.02 fmol/μL; GDX-E: range of 0.13-0.18 fmol/μL, mean
Figure 2. Representative serial micrographs (A, B) showing the placement of a microdialysis probe in the motor cortex of a gonadectomized animal. Cytoarchitectonic fields and boundaries between cortex and white matter are marked with dashed lines in the unimplanted hemisphere, damage caused by the probe itself is circled in the other hemisphere, and the modest damage in the contralateral hemisphere caused by an anchoring screw is marked with an asterisk. Line drawings in panels (C) and (D) (modified from Paxinos and Watson [1998]) mark the locations of microdialysis probe tracks (thick black lines) for each animal in the 4-day (panel C) and 28-day (panel D) groups. For both the 4-day and 28-day cohorts, probes were comparably placed with respect to cortical cytoarchitecture (dashed lines) in rats that were sham operated (CTRL) and gonadectomized (GDX). The number that appears in parentheses below the drawings identifies the number of animal subjects in the group. Cg1, anterior cingulated cortex; Prl, prelimbic cortex; IL, infralimbic cortex; DP, dorsopeduncular cortex; olf, olfactory bulb; wm, white matter.

0.15 ± 0.01 fmol/μL, see Fig. 4 A). Statistical evaluation (ANOVA) of these data further identified significant main effects of hormone treatment on basal PFC DA level ($F_{3,15} = 4.168, P = 0.025$), and post hoc comparisons confirmed that basal PFC DA values in the GDX-TP, GDX-E, and CTRL groups were all similar and not significantly different from one another and that the extracellular PFC DA level in the GDX rats was significantly lower than those of the other treatment groups (vs. CTRL, $P < 0.0094$; vs. GDX-TP, $P < 0.0086$; vs. GDX-E, $P < 0.0254$). Because this effect was attenuated by replacement with both testosterone and estrogen and thus included animals with both higher and lower BSM weights, regression analyses assessing animals' individual and average measures of extracellular PFC DA level as a function of BSM weight found no significant correlations (individual: $R^2 = 0.088$, $P < 0.108$ average: $R^2 = 0.115$, $P < 0.0856$) between PFC DA and androgen-sensitive muscle weight (Fig. 4 B).

28-Day Treatment Groups
Basal, extracellular DA levels measured in the 28-day CTRL group were similar to those obtained in the 4-day CTRL rats and to DA level reported in previous in vivo microdialysis studies of PFC in adult male rats (Moghaddam and Jackson 2004; Stefani and Moghaddam 2005; Del Arco et al. 2007; van der Meulen et al. 2007; Balla et al. 2009); these values were between 0.08 and 0.18 fmol/μL and were on average 0.13 ± 0.04 fmol/μL. Basal DA level measured in the 28-day GDX cohort, however, was nearly twice as high as control, ranging from 0.18 to 0.30 fmol/μL and averaging 0.23 ± 0.02 fmol/μL. Similarly elevated DA level was also observed in GDX-E rats (range of 0.16-0.27 fmol/μL, mean 0.22 ± 0.02 fmol/μL, see Fig. 5 A) while the values in the GDX-TP rats were similar to control (range of 0.09-0.15 fmol/μL, mean 0.12 ± 0.01 fmol/μL, see Fig. 4 C). ANOVA performed on these data identified significant main effects of hormone treatment on mean PFC DA level ($F_{3,24} = 9.197, P = 0.0003$), and allowed post hoc comparisons confirmed that basal PFC DA level in the GDX and GDX-E but not the GDX-TP group was significantly higher than control (CTRL vs. GDX, $P < 0.0017$; CTRL vs. GDX-E, $P < 0.0116$). In keeping with the selective androgen sensitivity of the GDX effect, regression analyses assessing animals' individual and mean measures of extracellular PFC DA level as a function of BSM weight further identified significant correlations between both measures of PFC DA level and androgen-sensitive muscle weight (individual: $R^2 = 0.249$, $P < 0.001$; average: $R^2 = 0.528$, $P < 0.001$, see Fig. 4 D).

Extracellular Motor Cortex Dopamine Level
For comparison, the effects of GDX were also assessed in a second DA-enriched area of the rat cerebrum—the motor cortical fields. However, in both 4- and 28-day sham-operated control and GDX rats alike, basal DA levels in this region were all between 0.09 and 0.23 fmol/μL and had mean values that ranged from 0.14 to 0.17 fmol/μL (Fig. 5 A, B). ANOVA found no significant main effects of hormone treatment on these motor cortex measures, and regression analyses assessing animals' individual and mean measures of extracellular sensorimotor DA level as a function of BSM weight revealed no significant correlations between motor cortical DA level and androgen-sensitive muscle weight (4 day: $R^2 = 0.029$, $P < 0.314$; 28 day: $R^2 = 0.038$, $P < 0.502$, see Fig. 5 C, D). Accordingly, no assessments of motor cortex were made in GDX hormone-supplemented animals.
Discussion

As evidence for the impact of gonadal steroid hormones on the development, adult performance, and the influence of disease of the highest order cognitive, affective, and mnemonic functions of the PFC grows, efforts to understand how this modulation might occur are expanding. The studies presented here follow up on findings, suggesting that in males the function of the PFCs may be modulated via gonadal steroid actions on their essential mesoprefrontal DA innervation (Janowsky et al. 2000; Kritzer et al. 2001; Gibbs 2005; Muller et al. 2005; Kritzer et al. 2007; Aubele et al. 2008). For example, these studies have foundations in previous anatomical studies from this laboratory showing striking effects of both long- and short-term GDX on medial prefrontal DA innervation density (Adler et al. 1999; Kritzer 2000), extracellular PFC DA level following GDX. Further, the differential consequences that supplanting GDX animals with estrogen versus testosterone has for PFC DA levels and axon density are also in keeping with the many examples of opposing influences that have been found for ovarian versus testicular hormone/intracellular receptor interactions in brain and peripheral tissues (Toran-Allerand 1991; Stewart and Rajabi 1994; Handa et al. 1997; Cutolo and Wilder 2000; Zhang et al. 2000; Krause et al. 2006). While the question of how they arise remains pure speculation, it is clear that the initial, short-term effects of GDX on PFC DA systems including those identified in this study are transient while those that are observed later at 28 days post-GDX appear to be more stable. Because effects at this longer time point also represent the condition where behavioral effects of GDX on DA-dependent prefrontal functions have been found (Adler et al. 1999; Kritzer et al. 2001, 2007; Aubele et al. 2008), discussion in the sections that follow focus mainly on results pertaining to the 28-day animal groups. The striking and seemingly PFC-selective effects of GDX on DA level that are seen at this stage are first compared with results from previous studies of sex differences and/or hormone effects on cortical DA concentration are then discussed in terms of possible mechanism and are finally considered with respect to the sex differences and hormone modifiability that are known to characterize information processing in the PFC of animal models and in humans in health and disease.

Hormone Effects on Prefrontal Cortical DA Level: Comparison to Previous Studies

Although numerous studies have found evidence for sex differences, estrus cycle variance and/or gonadal hormone regulation of DA in the rat CNS, the majority of these
investigations have focused on hypothalamic (Simpkins et al. 1983; Toney and Katzenellenbogen 1986), mesostriatal (Glick et al. 1983; Camp et al. 1986; Camp and Robinson 1988; Di Paolo et al. 1988; van Haaren and Meyer 1991; Becker and Rudick 1999; Walker et al. 2006), or mesolimbic (Vermes et al. 1979; Siddiqui and Gilmore 1988; Kuhn et al. 2001; Walker et al. 2001; Parylak et al. 2008) DA systems and on endpoints such as DA receptor binding, uptake, and/or stimulated release. On the other hand, among the relatively few assessments that have investigated sex differences and/or hormonal regulation among mesocortical DA systems are several that focus on endpoints similar to those examined here, namely whole tissue or extracellular cortical DA level.

One such study examining the effects of postnatal handling on DA level found that among the unhandled controls, DA concentrations in ventral mPFC and insular cortex homogenates were nearly 2-fold higher in female compared with male rats (Duchesne et al. 2009) while a second study using in vivo microdialysis showed that extracellular DA levels in the mPFC were significantly higher in female rats in estrus compared with proestrus, were significantly lower in ovariectomized (OVX) compared with intact animals, and were restored to near control level in OVX animals supplemented with E but not progesterone (Dazzi et al. 2007). There have also been 2 prior studies that examined gonadal hormone impact on cortical DA level specifically in male rats. The first showed that DA and DOPAC levels measured in parietal cortex homogenates were significantly higher in long-term GDX than control rats, although a 2-day TP replacement regimen did not attenuate these effects (Battaner et al. 1987). The second study compared levels of DA, DOPAC, and the other major DA metabolite, HVA in mPFC homogenates of control, 21-day GDX, and 21-day GDX adult male rats supplemented with dihydrotestosterone propionate or with E that did or did not also experience a novel environment immediately prior to euthanasia (Handa et al. 1997). Among the home-caged controls, this study found no differences in DA, DA metabolites, or their ratios between CTRL, GDX, or GDX hormone-replaced subjects. The discrepancies between these negative findings and those presented here could be due to the relatively subtle differences in duration of GDX, the dose and duration of E treatment, and/or the use of aromatizable versus nonaromatizable androgens. However, it may be more likely that the critical difference is that the prior mPFC study assessed whole tissue levels of DA measured from cortical

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**Figure 4.** Bar graphs showing mean extracellular DA level (fmol/μL) ± standard error of the mean in 4-day (A) and 28-day PFC (B) measured in animals that were sham operated (CTRL, black bars), gonadectomized (GDX, white bars), gonadectomized and supplemented with TP (GDX-TP, dark gray bars), or gonadectomized and supplemented with E (GDX-E, light gray bars). Mean DA level in the PFC was significantly lower in the GDX compared with CTRL rats and was similar to control in the GDX-TP and GDX-E groups in the 4-day animals. However, in the 28-day cohort, mean DA level in the PFC was significantly higher in the GDX and GDX-E compared with CTRL rats and was similar to CTRL in the GDX-TP group only. Regression plots that relate 20-min bin measurements of individual animals’ level of extracellular DA to the weights of their androgen-sensitive BSMs in grams (g) reveal no significant correlation between these 2 measures in 4-day rats (C) but that DA level and BSM weights are significantly correlated in 28-day animals (D). Open circles in the regression plots identify data points from GDX animals, and R² values from the regression analyses appear in the upper left.
homogenates which sum DA’s intracellular and extracellular pools, whereas the present study used in vivo microdialysis, which exclusively reflects extracellular DA level. Thus, rather than a disparity, it is possible that the data collected across these 2 studies both support the argument that the effects of GDX on the mesofrontal DA system specifically involve an exaggerated availability of DA in the prefrontal cortical extracellular space. As discussed further below, this scenario may gain some support from additional effects that GDX and hormone replacement have recently been shown to have on one player in the regulation of extracellular DA level within the mPFC.

**Hormone Effects on Prefrontal Cortical DA Level: Possible Mechanisms**

Behavioral and other studies have repeatedly shown that the cognitive, mnemonic, affective, and executive functions of the PFC rely on DA level being maintained within certain limits (Tassin et al. 1978; Kessler and Markowitsch 1981; Kalsbeek et al. 1989; Murphy et al. 1996; Verma and Moghaddam 1996; Zahrt et al. 1997; Morrow et al. 2000). In addition to showing that both too much and too little DA in the extracellular space can adversely affect PFC function, previous information from in vivo microdialysis (Finlay and Zigmond 1997; Watanabe et al. 1997) and electrophysiological studies (Lavin et al. 2005) have shown that DA’s essential contributions to prefrontal cortical function also require an unusually extended extracellular lifetime of this transmitter once it is released (Garris et al. 1993; Cass and Gerhardt 1994; Garris and Wightman 1994). In contrast to the more rapid signaling that is characteristic of the subcortical DA systems (Moghaddam 1993; Goto and Grace 2007), DA actions in the PFC are more paracrine in nature, that is, they rely in part on volume transmission (Garris and Wightman 1994; Zoli et al. 1998; Mundorf et al. 2001) owing in part to the minimal use of the dopamine transporter (DAT) and the norepinephrine transporter (NET) in clearing clear DA from the PFC synaptic space (Sesack et al. 1998a, 1998b; Miner et al. 2003; Shen et al. 2004) and instead a greater reliance on DA’s extracellular inactivation largely via the degradative
enzyme catechol-O-methyltransferase (COMT) (Karoum et al. 1994; Yavich et al. 2007). However, gonadal hormone regulation/GDX dysregulation of this enzymatic catalytic pathway is unlikely to contribute to the elevated extracellular DA level observed here as quantitative analyses of the O-methylation activity of both the soluble and membrane-bound forms of COMT in PFC homogenates found no significant differences in either enzyme isoform among 4- and 28-day sham-operated, GDX and GDX hormone-replaced adult male rats (Meyers et al. 2010). An influence on the DAT is also unlikely as, in vitro binding assays performed on PFC homogenates using the DAT-selective ligand \(^3\)H WIN 35 428 also showed that neither the K_d nor the B_{max} for this binding site was affected by 4- or 28-day GDX or hormone replacement. On the other hand, results from parallel analyses using the NET-selective ligand \(^3\)H nisoxetine did find significant, TP- but not E-sensitive effects of GDX on lowering the affinity of ligand binding to the NET (Meyers and Kritzer 2009). However, the magnitude of this potentially DA elevating effect might well be expected to be the modest given the limited roles that reuptake and uptake mechanisms play in the control of PFC DA tone overall. Rather, in view of the stimulatory effects that long-term GDX has on the density of PFC axons that are immunoreactive for the DA-synthesizing enzyme tyrosine hydroxylase (REF) and given the numerous precedents set for hormone effects on DA release in subcortical centers (Castner et al. 1993; Becker and Rudick 1999; Becker 2000; Dluzen and McDermott 2008), perhaps, more significant contributions to the anomalous extracellular PFC DA levels that are seen could come from GDX-induced dysregulation of DA synthesis and release.

**Summary and Conclusions**

There is growing evidence from clinical studies and animal models, suggesting that androgens influence DA-dependent PFC function in males (Christiansen and Knussmann 1987; Gouchie and Kimura 1991; Ceccarelli et al. 2001; Kritzer et al. 2001, 2007; Daniel et al. 2003; Janowsky 2006; Thilers et al. 2006; Aubele et al. 2008). The present results now suggest that this may be a consequence of androgen’s regulation of the functionally pivotal parameter of PFC extracellular DA level. Thus, while it had been previously shown that long-term GDX and hormone replacement with androgen selectively influences PFC DA axon density (Kritzer et al. 1999; Kritzer 2000) in a manner that is in parallel with and significantly correlated to GDX effects on DA-dependent PFC function/behavior (Kritzer et al. 2007), the present findings show that GDX also significantly and selectively increases extracellular PFC DA level or tone—again, in an androgen-sensitive, estrogen-insensitive manner. It is thus tempting to consider that too much PFC DA may be a large part of what produces the observed behavioral deficits in GDX animals and to speculate further that this new role for gonadal steroid and especially androgen hormones in adult male rats could help to explain and perhaps ultimately remedy the greater vulnerability of the mesoprefrontal cortical DA systems and DA-dependent PFC processes in males afflicted with disorders such as schizophrenia and attention deficit hyperactivity disorder (Goodman and Stevenson 1989; Seeman and Lang 1990). Accordingly, while previously observed androgen-sensitive effects of GDX on NET ligand binding could make some contribution to the elevated extracellular DA level observed here, it will be important to continue to characterize both the causes and the effects of hormone stimulation on the PFC DA systems in vivo. Impetus for this effort comes in part from the hope that such new information could hold for informing on-going strategies for using hormone treatments as adjunct therapies in the treatment of mental illness, including the specific use of testosterone in the treatment of male schizophranics that has already begun to show therapeutic promise in combating the negative and cognitive problems associated with this disorder. (Ko et al. 2008).

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