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Research report

Prolonged androgen deprivation may influence the autoregulation of estrogen receptors in the brain and pelvic floor muscles of male rats

Erik Wibowo[a,b], Hannah J. Calich[a], R. William Currie[a], Richard J. Wassersug[a,c,d,*]

[a] Department of Medical Neuroscience, 5850 College Street, PO Box 15000, Halifax, NS B3H 4R2, Canada
[b] Vancouver Prostate Centre, Vancouver General Hospital, 2775 Laurel St., University of British Columbia, Vancouver, BC V5Z 1M9, Canada
[c] Department of Urologic Sciences, Gordon & Leslie Diamond Care Centre, 2775 Laurel St., University of British Columbia, Vancouver, BC V5Z 1M9, Canada
[d] Australian Research Centre in Sex, Health and Society, La Trobe University 215 Franklin Street, Melbourne, Victoria 3000, Australia

HIGHLIGHTS

- Long-term castration increases estrogen receptor (ER) α in the preoptic area.
- Estradiol (E2) downregulates ERα in the preoptic area of Long-Term castrates.
- ERβ autoregulation may play a role in activating mounting behavior.
- E2 autoregulates ERs differentially in the hippocampus and prefrontal cortex.
- E2 only upregulates ERs in the pelvic floor muscles of early castrates.

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ABSTRACT

Androgen deprivation in males has detrimental effects on various tissues and bodily functions, some of which can be restored by estradiol (E2) administration. We investigated how the duration of androgen deprivation affects the autoregulation of estrogen receptors (ERs) levels in core brain areas associated with sexual behavior and cognition, as well as in pelvic floor muscles (PFM). We also measured c-Fos levels in brain areas associated with sexual behavior shortly after the rats mated.

Prolonged castration increases ERα levels in the preoptic area (POA) and E2 treatment reverses these effects. In the POA, c-Fos levels after mating are not affected by the duration of androgen deprivation and/or E2 treatment. ERβ levels in the POA as well as c-Fos levels in the POA and the core area of nucleus accumbens correlate with the mounting frequency for E2-treated Short-Term castrates. Additionally, ERβ levels in the medial amygdala are positively correlated with the mounting frequency of Long-Term castrates that received E2 treatment. In the hippocampus, ERs are downregulated only when E2 is administered early after castration, whereas downregulation of ERα in the prefrontal cortex only occurs with delayed E2 treatment. Early, but not delayed, E2 treatment after castration increases ERβ levels in the bulbocavernosus and ERα levels in the levator ani of male rats.

Our data suggest that the duration of androgen deprivation may influence the autoregulation of ERs by E2 treatment in select brain areas and pelvic floor muscles of male rats.

1. Introduction

Androgen deprivation therapy is prescribed to some men, for example, as part of prostate cancer treatment or sex-reassignment. Androgen deprivation, either by surgical or chemical means, depletes plasma testosterone levels and subsequently estradiol (E2) levels. The suppression of gonadal hormones in males has detrimental effects on various tissues and functions. However some of those adverse effects can be alleviated with estrogen (E) treatment [1–3].

At the cellular level, E2 must bind to estrogen receptors (ERs) in order to exert its effects. In response to E treatment, ERs autoregulate (either upregulate or downregulate) their expression to maintain normal physiological function [4]. Several types of ERs have been identified, including the nuclear receptors ERα and ERβ, and the membrane receptor GPR30 [5]. Studies in female rodents

* Corresponding author at: Department of Urologic Sciences, Gordon & Leslie Diamond Care Centre, 2775 Laurel St., 6th Floor, University of British Columbia, Vancouver, BC V5Z 1M9, Canada. Tel.: +1 604 875 4111x62338; fax: +1 604 875 5024.
E-mail addresses: erik.wibowo@vch.ca (E. Wibowo), hannah.calich@gmail.com (H.J. Calich), R.William.Currie@dal.ca (R.W. Currie), richard.wassersug@ubc.ca (R.J. Wassersug).

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suggest that the duration of hormone deprivation may alter the autoregulation of ERs in some brain areas [6,7]. In the present study, we explore how the duration of androgen deprivation (done via surgical castration) influences the autoregulation of ERs in brain areas that are involved in sexual behavior and cognition, as well as pelvic floor muscles (PFM) of male rats. ERs are expressed in these tissues [8–11], suggesting that E may modulate their functions.

In addition to the presence of ERs in these tissues, they are of interest because: (1) E may stimulate mounting behavior in male rats by acting on ERs in brain areas that are activated during a sexual encounter [12,13], (2) in females, E can improve cognitive function when administered early, but not late after hormone-deprivation (e.g., ovariectomy or menopause) [14,15], and (3) E has been shown to influence some visceral functions associated with PFM (notably, E therapy may help improve incontinence in women; [16,17]). Our results, we reasoned, would establish whether the length of time between castration and E2 administration in males affects the responsiveness of these tissues to E2, which may potentially influence their functions.

The tissues used in this study were collected an hour after the rats had a sexual encounter (behavior data previously published in Wibowo and Wassersug [3]). Given this time interval—optimal to demonstrate neuronal activation—we also examined how the duration of androgen deprivation with or without E2 administration affects the levels of c-Fos (as a marker for neuronal activation) in brain areas essential for sexual behavior. As additional analyses, we performed correlation analyses on the number of mounting behavior of the rats (from our previous behavioral study [3]) with ERs and c-Fos levels in these brain areas.

2. Materials and methods

2.1. Animals

The tissues used in this study were collected from the rats used in our previous behavioral experiment [3], and the experimental design, surgery and treatment protocols are only described briefly here. Adult male sexually naïve Long-Evans rats (Charles River Canada, Saint Constant, QC, Canada, 275–300 g at the time of arrival) were housed singly under a reversed 14:10 light:dark cycle (lights on at 1930 h) at 23 ± 1 °C ambient temperature, with food and water available ad libitum. Animal handling protocols followed the guidelines of the Canadian Council on Animal Care and were approved by the Dalhousie University Committee on Laboratory Animals.

Prior to castration, each rat received four weekly staged sexual encounters (30-min each) with an estrus female rat starting 1 week after arrival. Only male rats that ejaculated 2 or 3 times in their fourth sexual encounter were used to ensure that no hyper- or hypo-sexual rats were included. A total of 48 male rats met this inclusion criterion.

2.2. Surgery and oil/estradiol administration

Male rats were subjected to bilateral scrotal orchiectomy under anesthesia (104 mg/kg ketamine, 4.8 mg/kg xylazine, and 0.9 mg/kg acepromazine, intraperitoneal). After surgery, the rats were given an analgesic (5 mg/kg, ketoprofen, subcutaneous) and an antibiotic (Baytril, 5 mg/kg, subcutaneous), then returned to the animal care facility for recovery.

The castrated rats were randomly divided to receive either sesame oil (as a control) or E2 (dissolved in sesame oil) treatment ($n = 24$ per treatment group). Animals in the same treatment group were further assigned randomly into three groups ($n = 8$ per group) according to the timing at which a Silastic tube (1.6 mm inner diameter, 3.2 mm outer diameter, 35 mm in length; Dow Corning Corporation, Midland, MI) was implanted: immediately (Immediate), 1 month (Short-Term), or 3 months (Long-Term) after castration. The 1-month interval for the Short-Term groups was chosen because within 1 month of castration approximately 90% of rats have impaired sexual functions [18]. In addition, the 3-month interval for the Long-Term groups was chosen because 6–7 weeks of hormone deprivation in female rats was shown to dampen the restorative effect of E2 on their sexual behavior [19,20]. Extending that to 3-months ensured that Long-Term groups had been androgen-deprived for a prolonged period before receiving treatments.

Each Silastic tube was filled with either sesame oil (60 μL; Catalog No. S3547, Sigma–Aldrich) for the oil groups, or 230 μg of 17β-E2 (Catalog No. E8875; Sigma–Aldrich) in 60 μL of sesame oil for the E2 groups. This E2 dose raised the plasma E2 levels of the castrated male rats to levels similar to those of proestrus female rats [2,3]. Although these were supra-physiological levels for normal male rats, those levels were chosen because of their clinical relevance to human males receiving exogenous E to treat various medical conditions. Those include E therapy both to suppress testosterone in prostate cancer patients [21,22] as well as to treat male-to-female transsexualism [23].

Rats in the Short-Term and Long-Term groups received their Silastic implants under isoflurane anesthesia (4% for induction, 2% for maintenance; 1 L/min) mixed with oxygen. The Silastic implant size, oil volume, and E2 dose were the same as those used with the Immediate groups. After castration, the male rats did not have further sexual encounters with female rats until the final behavioral test, which occurred at 2 weeks after the Silastic tube implantation.

2.3. Tissue collection

One hour after the final behavioral test, the male rat was euthanized by anesthetic overdose (208 mg/kg ketamine, 9.6 mg/kg xylazine, and 1.8 mg/kg acepromazine, intraperitoneal). The brain was quickly removed and frozen at −80 °C.

Two PFM were also collected at that time, the bulbocavernosus (BC) and levator ani (LA). Both muscles from each side in each rat were quickly dissected out and separated from each other. Muscle tissues were frozen at −80 °C until they were used for Western analysis.

2.4. Western blot

To collect data from specific brain areas, the brains were cut into 300-μm thick sections using a cryostat (Leica CM1850) at temperatures between −7 and −10 °C. Brain areas of interest were micropunched according to the techniques described by Palkovitz and Brownstein [24]. The following brain areas associated with sexual behavior [25,26] were examined: preoptic area (POA), bed nucleus of the stria terminalis (BNST), medial amygdala (MeA), the shell (NAs) and core (NAc) regions of nucleus accumbens. The following brain areas associated with cognitive function [27] were also examined: prefrontal cortex (PFC) and hippocampus (HC).

The BNST, PFC, NAs and NAc were sampled using a 1.0 mm Harris MicroPunch™ (Catalog No. 69035-10, Electron Microscopy Sciences). The POA and MeA were sampled using a 0.5 mm Harris MicroPunch™ (Catalog No. 69035-05, Electron Microscopy Sciences). The entire HC, including both the dorsal and ventral HC, was microdissected from each rat. Each brain tissue sample was immediately placed into either 25 μL (POA, MeA), 40 μL (BNST, NAs, NAc, PFC) or 400 μL (HC) of homogenization buffer (0.32 M sucrose in 0.1 M phosphate-buffered saline with one complete mini protease inhibitor tablet (Catalog No. 04 693 124 001, Roche) per 10 mL).