Schizophrenia Research xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Comparing the effects of 17β-oestradiol and the selective oestrogen receptor modulators, raloxifene and tamoxifen, on prepulse inhibition in female rats

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ARTICLE INFO

Article history:
Received 2 December 2014
Received in revised form 12 April 2015
Accepted 23 April 2015
Available online xxxx

Keywords:
SERMs
Raloxifene
Tamoxifen
17β-Estradiol
Prepulse inhibition (PPI)
Female rats

ABSTRACT

Background: Evidence suggests that oestrogen plays a protective role against the development and severity of schizophrenia. However, while oestrogen may be beneficial as a treatment in schizophrenia, its chronic use is associated with side-effects. Selective oestrogen receptor modulators (SERMs) may provide an alternative, however little is known about the mechanism underlying their effects in schizophrenia.
Methods: We investigated the effect of raloxifene and tamoxifen on dopaminergic-induced disruptions of prepulse inhibition (PPI). PPI measures sensorimotor gating and PPI disruptions are considered an endophenotype for schizophrenia. Adult female Sprague–Dawley rats were either intact, ovariectomized (OVX), OVX and 17β-oestradiol-treated, OVX and raloxifene-treated (low or high dose), or OVX and tamoxifen-treated (low or high dose).
Results: The dopamine D_1/D_2 receptor agonist, apomorphine (0, 0.1, 0.3 and 1 mg/kg), caused the expected dose-dependent disruption in PPI in intact and OVX rats. This PPI disruption was prevented in OVX rats treated with 17β-oestradiol, a high dose of raloxifene or a high dose of tamoxifen. In untreated OVX rats, average PPI was 55% after saline and 34% after 1 mg/kg apomorphine treatment, a reduction of 21%. However, oestradiol-treated and raloxifene-treated OVX rats showed only a 7% PPI reduction, and tamoxifen-treated OVX rats had a 4% PPI reduction caused by apomorphine treatment. Startle amplitude was not different between the groups.
Conclusion: The SERMs, raloxifene and tamoxifen, can prevent dopamine D_1/D_2 receptor-mediated disruptions of sensorimotor gating, similar to oestradiol. These data lend support for the use of SERMs as a treatment for schizophrenia.

1. Introduction

An extensive literature of epidemiological, clinical and animal studies has described how oestrogens play a role in a range of mental illnesses, including schizophrenia, bipolar disorder and depression. For example, gender differences in schizophrenia have suggested a role for oestrogen; men and women with schizophrenia differ in terms of incidence, age-at-onset, symptom severity and functional outcome (Häfner et al., 1993; Szymanski et al., 1995; Castle et al., 1998; McGrath et al., 2004; Canuso and Pandina, 2007). Further, lower levels of serum oestradiol are found in women with schizophrenia, with and without antipsychotic-induced hyperprolactinemia (Bergemann et al., 2005). These data have led to the suggestion that oestrogen plays a functionally 'protective' role against schizophrenia (Häfner et al., 1993; Seeman, 1996; Wu et al., 2013). Interestingly, two groups have conducted placebo-controlled trials in women with schizophrenia and found that adjunctive oestrogen treatment reduced positive and general psychopathological symptoms (Akhondzadeh et al., 2003; Kulkarni et al., 2008a).

Although oestrogen may be beneficial in treating schizophrenia, its chronic use is associated with side-effects, such as an increased risk of stroke and ovarian cancer (Anderson et al., 2004). Selective oestrogen receptor modulators (SERMs) may provide a better alternative to oestrogen and a safer treatment option for both men and women. SERMs, such as raloxifene, tamoxifen and bazedoxifene, are synthetic compounds which bind with high affinity to oestrogen receptors, however their action varies depending on the target tissue (Pinkerton and Thomas, 2014). Tamoxifen, is commonly used for prevention and treatment of breast cancer as it acts as an oestrogen receptor antagonist in the breast tissue (Littleton-Kearney et al., 2002). The second-generation SERM, raloxifene, acts as a partial oestrogen receptor agonist in the bone and an antagonist in the uterus and breast tissue, thus it is approved for the prevention and treatment of osteoporosis and

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http://dx.doi.org/10.1016/j.schres.2015.04.029
0920-9964/

invasive breast cancer, without increasing the risk of uterine cancer (Littleton-Kearney et al., 2002; Pinkerton and Thomas, 2014). Importantly, these synthetic compounds also act as oestrogen receptor agonists in certain brain regions (Littleton-Kearney et al., 2002).

Clinically, synthetic and natural forms of oestradiol have been found to influence cognitive function (Maki et al., 2002; Sherwin, 2012; Gogos, 2013; Gogos et al., 2014). Raloxifene has been found to improve verbal memory in post-menopausal women (Jacobsen et al., 2010) and in women with schizophrenia (Wong et al., 2003; Huerta-Ramos et al., 2014), and reduce the risk of cognitive impairment in post-menopausal women (Yaffe et al., 2005). Recent studies have used raloxifene as an adjunct to antipsychotic medication in post-menopausal women with schizophrenia and have found that it reduced positive, negative and general psychopathological symptoms (Kulkarni et al., 2010; Usall et al., 2011; Kianimehr et al., 2014). Tamoxifen has also been trialled as an adjunctive treatment for mental illness and was found to reduce mania in bipolar disorder and schizoaffective disorder (Kulkarni et al., 2008b; Amrollahi et al., 2011). Raloxifene and tamoxifen act on both oestrogen receptor subtypes (ERα and ERβ) (Littleton-Kearney et al., 2002), although their specific profiles differ depending on the brain region (Zhou et al., 2002). These SERMs also influence multiple transmitter systems. For example, raloxifene mimics the effect of oestrogen on serotonin transporter and 5-HT_{2A} receptor expression in ovariectomized animals (Cyr et al., 2000; Bethea et al., 2002). Tamoxifen increases extracellular dopamine levels in the striatum of freely moving rats (Chaurasia et al., 1998). However, in terms of their therapeutic benefit in schizophrenia, little is known about the mechanism of action of SERMs in the brain.

Prepulse inhibition (PPI) is an operational measure of sensorimotor gating or information processing, which is disrupted in schizophrenia (Braff et al., 2001) and in experimental animals after specific drug treatments (Geyer et al., 2001). Sex differences have been observed in PPI. For example, PPI was reduced in men with schizophrenia compared to healthy men, but women with schizophrenia did not differ from healthy women (Kumari et al., 2004) in line with the proposed 'protective' role of oestrogen in the illness. We have shown that the dopamine D_1/D_2 receptor agonist, apomorphine, the 5-HT_{1A} receptor agonist, 8-OH-DPAT, and the NMDA glutamate receptor antagonist, MK-801, can all disrupt PPI in female rats and that this disruption could be inhibited by treatment with oestradiol (Gogos et al., 2010, 2012). Similarly, we showed in healthy women, that the PPI disruption caused by treatment with the 5-HT_{1A} receptor partial agonist, buspirone, could be inhibited by treatment with oestrogen (Gogos et al., 2006). Our studies suggest an antipsychotic-like effect of oestrogen, similar to haloperidol (Gogos et al., 2010), via an action on downstream dopaminergic regulation. Indeed, autoradiography studies confirmed this, whereby oestradiol treatment reversed both the increase in dopamine D_2 receptor binding and the reduction in dopamine transporter caused by ovariectomy (Chavez et al., 2010).

Our previous research suggests that oestrogen may protect against mechanisms that disrupt PPI by acting on the dopaminergic system. However, it is unknown if a similar mechanism plays a role in the therapeutic effectiveness of SERMs in schizophrenia. The present study aimed to investigate the effect of oestradiol and SERMs (raloxifene and tamoxifen) in an animal model with relevance to schizophrenia: dopaminergic-induced disruptions of PPI.

2. Methods

2.1. Animals

We used 75 female Sprague–Dawley rats (Animal Resources Centre, WA, Australia) which were 12 weeks of age at the time of surgery. The animals were housed in groups of 3–4 in individually ventilated cages (Tecniplast Greenline Double Decker IVC), with free access to standard pellet food and tap water. The rats were maintained on a 12-h light–dark cycle (lights on at 0700h), at a constant temperature of 22 ± 2 °C. All surgical techniques, treatments and experimental protocols were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (1990) set out by the National Health and Medical Research Council of Australia.

2.2. Surgery

Ovariectomy surgery was performed as previously described (Gogos et al., 2010). Briefly, rats were anaesthetised using an isoflurane/oxygen gas mixture and placed upon a heat pad in the prone position. Rats received a subcutaneous (s.c.) injection of 5 mg/kg of the non-steroidal, anti-inflammatory analgesic, carprofen (Rimadyl®; Heriot AgVet, VIC, Australia). A 2–3 cm midline incision was made through the skin above the lower back, followed by an incision through the abdominal wall. The ovaries were bilaterally located and removed and the incisions closed. Intact rats were sham-operated; they were anaesthetised, received carprofen, had their skin and abdominal wall cut, but the ovaries were not removed.

During the ovariectomy procedure, rats received a s.c. silastic implant at the nape of the neck as previously described (Gogos and Van den Buuse, 2004). Briefly, these implants (Dow Corning, I.D. 1.98 mm, O.D. 3.18 mm; Futuremedics Australia, VIC, Australia) were either empty or filled with crystalline steroid hormones. Oestrogen implants were 5 mm long, and filled with 17β-oestradiol (~25 mg per implant; Sigma Chemical Company, MO, USA). Raloxifene hydrochloride and tamoxifen (Toronto Research Chemicals, ON, Canada) implants were either 10 mm (~50 mg per implant) or 2 × 25 mm (~120 mg per implant) in length. These implant sizes were based on the literature and our previous findings, and were aimed at producing pharmacologically active doses (Albert et al., 1991; McDermott et al., 1995; Gogos and Van den Buuse, 2004; Gogos et al., 2010; Walf and Frye, 2010; Velazquez-Zamora et al., 2012).

Behavioural experiments commenced two weeks after surgery and continued for 2–3 weeks. At least three days after completion of experiments, rats were euthanized using a lethal dose of pentobarbitone and then decapitated. The uterus was removed, weighed and inspected for any abnormalities. Uterus weight was used as an index of ovariectomy and hormone treatment effects. Rats were excluded if ovariectomy was incomplete or hormone implants had fallen out.

2.3. Behavioural experiments

Prepulse inhibition (PPI) of the acoustic startle response was measured with eight automated startle chambers (SR-Lab; San Diego Instruments, San Diego, CA, USA) as previously described (Gogos and Van den Buuse, 2003). Briefly, rats were placed individually into a transparent Plexiglas cylinder in a sound-attenuating cabinet. The PPI session comprised 80 trials presented with variable intervals (8–27 s), including 32 pulse-alone trials (4 blocks of 8 115 dB trials) and 40 prepulse–pulse trials. Prepulse–pulse trials consisted of a prepulse (PP) of an intensity of 2, 4, 8, 12 or 16 dB above the 70 dB background (8 of each), followed 100 ms later by the startle pulse. Startle data were measured using all 4 blocks of pulse-alone trials. The %PPI was calculated as [(pulse-alone trials startle response amplitude − prepulse–pulse trials startle amplitude) / (pulse-alone trials startle amplitude)] × 100%. The middle 16 pulse-alone trials were used to calculate %PPI. Four rats were excluded because of equipment failure or insufficient startle amplitudes.

2.4. Experimental protocol

Female rats were randomly chosen to become intact, sham-operated controls (n = 9), ovariectomised (OVX) rats receiving an empty implant (n = 9), OVX rats treated with 17β-oestradiol (E2, n = 11), OVX rats

Please cite this article as: Gogos, A., van den Buuse, M., Comparing the effects of 17β-oestradiol and the selective oestrogen receptor modulators, raloxifene and tamoxifen, on prepulse in..., Schizophr. Res. (2015), http://dx.doi.org/10.1016/j.schres.2015.04.029