G PROTEIN-COUPLED ESTROGEN RECEPTOR 1 (GPER) AS A NOVEL TARGET FOR SCHIZOPHRENIA DRUG TREATMENT

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Abbreviations:

BDNF - brain-derived neurotrophic factor
CaV - voltage-gated calcium channel
CNS - central nervous system
CREB - transcription factor cAMP response element-binding protein
CRHR - corticotropin-releasing hormone receptor
DA – dopamine
E2 - 17β-estradiol
EGFR - epidermal growth factor receptor
ER – estrogen receptors
ERα - estrogen receptor alpha
ERβ - estrogen receptor beta
ERK - extracellular-signal-regulated kinase
GPER1 – G protein-coupled estrogen receptor
HRT - hormone replacement therapy
IDO1 - indoleamine 2,3 dioxygenase 1
IL - interleukin
MAP - mitogen-activated protein
MPO - myeloperoxidase activity
NF-kB - nuclear factor kappa B
NO – nitric oxide
NOS - nitric oxide synthase
OCD - obsessive-compulsive disorder
PANSS - Positive and Negative Syndrome Scale
PFC – prefrontal cortex
PI3K - phosphatidylinositol 3-kinase
PKA - protein kinase A
PLC - phospholipase C
poly I:C - polyinosinic:polycytidylic acid
PTSD - post-traumatic stress disorder
SERMs - selective estrogen receptor modulators
TRPC3 - transient receptor potential 3
UHR - ultrahigh risk patients

Highlights

- Schizophrenia pathophysiology involves immunoinflammatory and oxidative alterations
- GPER agonism produces anti-inflammatory and antioxidant effects
- Estrogen, SERMS, and GPER agonists reduce SCZ symptoms
- Estrogen receptors have rarely been targets of antipsychotic drug development
ABSTRACT

The observation that a person’s sex influences the onset age of schizophrenia, the course of the disease, and antipsychotic treatment response suggests a possible role for estrogen receptors in the pathophysiology of schizophrenia. Indeed, treatment with adjunctive estrogen or selective estrogen receptor modulators (SERMs) are known to reduce schizophrenia symptoms. While estrogen receptors (ER$\alpha$ and ER$\beta$) have been studied, a third and more recently discovered estrogen receptor, the G protein-coupled estrogen receptor 1 (GPER), has been largely neglected. GPER is a membrane receptor that regulates non-genomic estrogen functions, such as the modulation of emotion and inflammatory response. This review discusses the possible role of GPER in brain impairments seen in schizophrenia and in its potential as a therapeutic target. We conducted a comprehensive literature search in the PubMed/MEDLINE database, using the following search terms: “Schizophrenia,” “Psychosis,” “GPER1 protein,” “Estrogen receptors,” “SERMs,” “GPER agonism,” “Behavioral symptoms,” “Brain Inflammation.” Studies involving GPER in schizophrenia, whether preclinical or human studies, have been scarce, but the results are encouraging. Agonism of the GPER receptor could prove to be an essential mechanism of action for a new class of “anti-schizophrenia” drugs.

Keywords: Schizophrenia; Estrogen; G Protein-coupled estrogen receptor 1; Estrogen receptors; Sex
Introduction

Kraepelin (1919) was the first to report sex influences in schizophrenia. He noted that the disorder was more frequent in men and that, compared to men, women showed a delay in the onset of the first cognitive symptoms and age at first hospitalization. Since then, evidence has accumulated showing that sex/gender influences the epidemiology, the symptomatic expression, the life course, and the response to antipsychotics in schizophrenia. Table 1 presents the main sex differences seen in schizophrenia.

Estrogens play a critical role in female physiology and protect against numerous diseases in premenopausal women. In addition, they regulate male reproductive and nonreproductive organs. Based on the protective effects of estrogen observed in schizophrenia, the “estrogen protective hypothesis” was formulated. The hypothesis predicted that psychotic symptoms in women worsen at times in the menstrual cycle when estrogen levels are low, i.e., around the time of menstruation. This hypothesis continues to be tested in preclinical and clinical studies because the neurobiological mechanisms that might underlie it are still poorly understood.

What is generally acknowledged is that gene × sex (G×S) interactions are implicated in schizophrenia. For instance, polymorphisms of the catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO) genes, crucial to the metabolism of dopamine, the most relevant neurotransmitter in schizophrenia, are associated with schizophrenia in a sex-specific manner. A genome-wide association study (GWAS) has also found a clinical and molecular modulation by sex of the association between single nucleotide polymorphism (SNP) rs1344706 in the gene ZNF804A that encodes the Zinc finger protein 804A (related to the regulation of dendritic spine maintenance and neuron projection development), and risk of...
schizophrenia. Another study found an association between the polymorphism rs7597593 of the ZNF804A gene and quasi-psychotic experiences. Interestingly, the strength of the association was driven by female study participants. The ZNF804A gene is expressed throughout the brain, but especially in the developing hippocampus and the cortex. Another GWAS reported that the SLC30A3 gene (that encodes the protein zinc transporter 3) increases the risk of schizophrenia, but only in females.

Sex-specific effects can be mediated through the classical estrogen receptors (ERs) α and β, which are ligand-activated transcription factors that influence the function of several genes, an action referred to as genomic signaling. ERα and ERβ are also involved in rapid estrogen action (non-genomic signaling) when located in the plasma membrane of cells. For instance, in the brain, small amounts of ERα have been found in the plasma membrane of hypothalamic neurons and in the cornu ammonis (CA)1 hippocampal neurons. Both ERα and ERβ are also present in oligodendrocytes. Their presence in cellular plasma membranes, however, is relatively small, which suggests that they may not be entirely responsible for the non-genomic actions of 17-β estradiol.

In the 1990s, an orphan receptor designated as GPR30 was cloned. By 2000, it became clear that GPR30 expression is necessary for the “rapid”/“non-genomic” membrane-associated effects of estrogen. Initially believed to represent a G protein-coupled receptor for cytokines, the functions of GPR30, renamed GPER (G protein-coupled estrogen receptor) by the International Union of Basic and Clinical Pharmacology (IUPHAR) in 2007, have remained elusive until recently.

It is now known that GPER has neuroprotective properties and that it facilitates social cognition, learning, and memory. GPER modulates estrogenic actions at
synapses in the rat hippocampus, influencing neurite outgrowth, and glial cell function. Due to its presence in the hippocampus, GPER has been implicated in memory formation. With respect to biological processes, GPER modulates apoptosis, the cell cycle process, cell differentiation, immunity, the inflammatory response, innate immunity, and neurogenesis. Notably, cognitive deficit and social impairment are core behavioral complications of schizophrenia, whereas apoptotic mechanisms, and immuno-inflammatory alterations constitute important aspects of the pathophysiology of schizophrenia.

Therefore, based on the functions regulated by GPER and their close relation to schizophrenia pathophysiology, this review addresses the role of GPER as a putative target for schizophrenia treatment, with an emphasis on GPER’s modulatory effects on immune-inflammatory mechanisms. The rationale for this approach is based on what we know and are still discovering about the influence of a person’s sex on estrogen signaling pathways, and on the disease course of schizophrenia. We use the term “sex” to refer to biological differences between males and females. “Gender” which usually refers to the social roles and behaviors of men and women, is also a fundamental issue in the understanding of schizophrenia, but will not be explored in this review.
Table 1 – Summary of the main sex differences in schizophrenia

| Parameter                                | Men                        | Women                                      | Reference |
|------------------------------------------|----------------------------|--------------------------------------------|-----------|
| Age of onset (average)                   | Age 18                     | Two peaks: age 25 and age 45               | 28        |
| Response to antipsychotic treatment      | Higher doses required      | Lower doses required until menopause       | 2         |
| Side effects of antipsychotics           | More acute dystonias       | More weight gain                           | 29,30     |
|                                          |                            | More osteoporosis                          |           |
|                                          |                            | More agranulocytosis                       |           |
| Social function                          | Impaired                   | Less impaired                              | 31,32     |
| Symptoms                                 | More negative and cognitive symptoms | More affective and positive symptoms | 30,33     |
| Course and outcome                       | Worse course and outcome until middle age | More favorable course and outcome until middle age | 2,34     |
| Genetic antecedents and pre and perinatal risk factors | More vulnerable to early adversity; more “second hits” | Less vulnerable to early adversity; fewer “second hits” | 35,36 |
Search Strategy

We conducted a comprehensive literature search using the PubMed/MEDLINE database to identify studies relevant to this review. We used the following combinations of subject headings: “Schizophrenia” (MeSH) OR “GPER1 protein” AND “Estrogen receptors” (MeSH) OR “SERMS” (MeSH) OR “GPER1 agonism” (MeSH) OR “Behavioral symptoms” (MeSH) OR “brain inflammation” (MeSH). We included papers published in the English language up to August 2020. To improve our search strategy, we also inspected the reference lists of retained articles and tracked their citations in Google Scholar. Observational, experimental studies in both animal and human subjects and relevant literature reviews were included. The methodological quality of retrieved references played a decisive role in our choice of citations.

Estrogen genomic and nongenomic receptors, their interaction and putative relevance to advances in schizophrenia research

The classical nuclear (genomic) receptors ERα and ERβ, and the transmembrane receptor GPER mediate estrogen effects. GPER is present in both the plasma membrane and the endoplasmic reticulum and activates a non-genomic signaling pathway. Genomic receptors mediate long-term effects by involving gene transcription; GPER produces rapid effects by regulating intracellular signaling cascades.

Both ERα and ERβ are expressed in brain areas related to mood regulation, namely the prefrontal cortex (PFC), hippocampus, hypothalamus, and amygdala. In female rats, ERα is predominantly expressed in brain regions implicated in controlling reproductive functions, such as the hypothalamus and preoptic area. At the same time, in primates, ERα mRNA is found in abundance in the PFC. ERβ is expressed in the same brain regions as ERα, especially in the hippocampus of rodents and humans, indicating that these ERs are also
involved in regulating non-reproductive estrogen actions, such as learning and memory \(^{41}\) (Fig. 1). All ERs located outside of the nucleus seem to be responsible for the rapid actions of estrogens \(^{42}\). ER\(\alpha\) and ER\(\beta\) have also been shown to activate nongenomic signaling through signal transduction proteins \(^{39,43,44}\).

ER\(\alpha\) and ER\(\beta\) cannot, however, explain all of estrogen’s actions. For example, the activation of ER\(\alpha\) and ER\(\beta\) by 17\(\beta\)-estradiol is unable to explain the antioxidant effects of this hormone \(^{45}\). Facts such as these call attention to the importance of a better understanding of GPER’s role in the neurobiology of mental disorders such as schizophrenia, in which oxidating mechanisms are fundamentally involved \(^{46}\).

Importantly, there is evidence that GPER modulates nuclear ERs, amplifying or diminishing a cell’s response to estrogen (Please see \(^{47,48}\), for a review on this topic). This modulation may take place in a variety of ways: i) GPER may collaborate with the nuclear ERs, mainly ER\(\alpha\) \(^{49}\); ii) GPER may antagonize signaling by ER\(\alpha\) or ER\(\beta\) either by blocking their expression or their downstream signaling pathways. This has been observed, for example, when prostate stromal cells differentiate into cancer-associated fibroblasts \(^{50}\); and iii) GPER may not interact with ER\(\alpha\) or ER\(\beta\) but, instead, may mediate 17\(\beta\)-estradiol-driven output on its own, as can be seen in cells where GPER is the sole estrogen receptor, for example, in SKBR3 breast cancer cells \(^{51}\). An example of the parallel activation of GPER and ER\(\alpha\) is the modulation of the rise in extracellular excitatory post-synaptic potentials (EPSPs) in CA3-CA1 hippocampal synapses mediated by estradiol benzoate (EB) \(^{52}\). In this latter study, both EB and the GPER agonist, G1, increased the synaptic response to a similar extent. The prior administration of G1 blocked the EB-mediated enhancement of the synaptic response. GPER1 antagonism by G15 inhibits the enhancement of the synaptic response induced by EB, suggesting that GPER is a major source of this effect \(^{52}\).

Furthermore, in the dorsal striatum, ER\(\alpha\), ER\(\beta\), and GPER have been found exclusively at extranuclear sites and have sometimes been associated with cholinergic neurons, since they were found to be localized to profiles containing vesicular ACh transporter (VACHT), a marker of cholinergic neurons, but not with dopaminergic ones. Hence, binding to cholinergic
receptors must influence neurotransmission via nongenomic mechanisms. Hence, knowledge of co-localization and estrogen receptor interactions appear to be essential to understanding estrogen effects in schizophrenia. Such knowledge clarifies which brain areas and which neurotransmitters are relevant to this condition.

It is known that variants of ERα and its mRNA contribute to the risk for schizophrenia and that ERα mRNA levels are reduced in the dentate gyrus of patients with schizophrenia compared with control subjects. It is also known that ERα single-nucleotide polymorphisms (rs2234693 and rs9340799) decreased levels of central ERα, and ERβ mRNA, in the presence of generally low overall level of estrogens, disrupt estrogen signaling in the brain of patients with schizophrenia. GPER polymorphisms associated with schizophrenia are, to date, unknown.

One important observation about GPER that is relevant to schizophrenia is that sex and age influence its expression and function. Accordingly, the GPER agonist, G1, increases EPSPs in hippocampal slices obtained from ovariectomized ERα knockout (ERαKO) and ERβ knockout (ERβKO) mice. GPER-induced potentiation of excitatory synaptic responses in CA1 hippocampal pyramidal neurons involves postsynaptic mechanisms and seems restricted to females. Furthermore, gene expression of GPER is significantly higher in the adult female hypothalamus than in the adult male hamster. In contrast, the opposite expression pattern was observed in the thalamus.

Similarly, the expression pattern of GPER mRNA displayed a contrary male/female trend in the cerebellum and amygdala of young hamsters. GPER agonism shows age-dependent protective effects in male and female rodents in animal models of cardiac diseases. This agonism protects the hearts of old mice of both sexes and young females but not young males. Our research group's previous study showed that adult female rats exposed to the two-hit model of schizophrenia (based on neonatal exposure to a viral mimetic and peripubertal unpredictable stress) showed decreased hippocampal expression of GPER, a decrease not observed in males.
With respect to humans and estrogenic effects, a second incidence peak of schizophrenia is recognized as occurring in women around the time of menopause when estrogen levels fall. Furthermore, psychotic symptoms, such as hallucinations and delusions, worsen in women as they approach menopause. They often require increased antipsychotic doses at this time. In contrast, in men of the same age, psychotic symptoms generally improve. Such observations suggest that the interaction among estrogen receptors needs to be studied in animal models of schizophrenia, both male and female and of different ages. GPER and its interaction with other ERs throughout the estrous cycle of females also needs to be investigated in order to understand the neurobiological mechanisms underlying perimenstrual susceptibility to increased levels of psychosis.

Because of the relevance of estrogen signaling to schizophrenia and knowing that other reviews have addressed the role of ERα and ERβ in schizophrenia, in the next sections, we focus on GPER in order to determine its putative role in schizophrenia neurobiology.

**GPER – The Transmembrane Estrogen Receptor: Functions and Cellular Mechanisms**

GPER was identified in the late 90s by multiple research groups. It was first named GPR30, an orphan receptor designation. In 2000, Filardo and his team demonstrated that GPER was required for estrogen-mediated activation of ERK1/2 and cAMP generation. Current evidence indicates that GPER promiscuously couples to both Gi/o and Gs, being this mechanism cell line and tissue-specific. GPER was found responsible for rapid non-genomic estrogen effects in the reproductive, nervous, endocrine, immune, and cardiovascular systems (although small effects on gene expression have also been shown). This receptor is present in the striatum, prefrontal cortex, hypothalamus, anterior and posterior pituitary, and brainstem of male and female rats.

Moreover, GPER has been detected in the rat forebrain (both pre- and post-synaptic sites) and the mouse hippocampus. Bruce McEwen and his group found that the receptor modulates estrogenic actions at synapses in the rat hippocampus and is partially responsible...
for neurite outgrowth and glial cell function. GPER has the ability to associate with other receptors coupled to post-synaptic G proteins, such as the corticotropin-releasing hormone receptor (CRHR) and the serotonin (5HT1a) receptor. It can also bind to a synapse-associated protein, postsynaptic density protein-95 (PSD95). This binding of GPER to PSD95 increases the plasma membrane levels of this receptor. Due to its synaptic location, GPER has been implicated in activity-dependent synaptic plasticity that is induced at appropriate synapses during memory formation in the hippocampus. Based on the brain localization of GPER and on the intracellular mechanisms regulated by this receptor, we next explore its involvement in memory and behavior regulation.

**GPER in the regulation of cognition and behavior**

GPER can be found in basal forebrain cholinergic neurons, important for cognitive control. The best evidence for the behavioral effects of GPER comes from animal studies indicating its involvement in spatial memory and social behavior in females. Experiments using a GPER agonist, G-1, show an improvement of spatial memory in pre-treated ovariectomized rats on the Y-maze task. By contrast, the GPER antagonist, G-15, has been shown to impair working memory in a delayed matching position task in ovariectomized rats. These effects may be mediated by the release of neurotransmitters and the growth of new dendritic spines on hippocampal neurons. G-1 post-training infusion in the hippocampus of ovariectomized mice increases object recognition and spatial memory, in contrast to G-15, which impairs spatial memory.

Social learning skills in female rodents are rapidly improved after treatment with a GPER agonist. This conclusion is based on the study by Ervin and colleagues (2015) in which they used G-1 in mice subjected to a social transmission of food preference task. In this task, a mouse observes a conspecific eating and thereby develops a preference for a particular flavor. In this study, the GPER-agonist G-1 favored social learning, while ERα, ERβ agonists did not have any effect. One proposed mechanism for social recognition regulation involves an interaction between ERβ and GPER that controls hypothalamic
oxytocin release and oxytocin receptor activation in the amygdala. Oxytocin mechanisms are the main regulators of social recognition in females, while in males, this function is primarily regulated by arginine-vasopressin \(^{72}\). In a recent study conducted by our research group in rats of both sexes, we observed working memory deficits only in female rats exposed to a two-hit model of schizophrenia induced by neonatal exposure to the viral particle polynosinic:polycytidylic acid (poly I:C) plus peripubertal unpredictable stress. The working memory deficits we found were accompanied by decreased hippocampal expression of GPER \(^{73}\).

Besides psychotic and cognitive symptoms, individuals with schizophrenia also suffer from anxiety. Anxiety symptoms are present in up to 65 % of patients with schizophrenia \(^{74}\). The role of GPER in anxiety-like behaviors in experimental animals is still not clear \(^{75}\) but a novel study conducted in GPER-deficient rats demonstrated that this receptor is widely distributed along the hypothalamic-pituitary-adrenal (HPA) axis. Genetic ablation of GPER resulted in lowering the basal serum corticosterone level but enhancing adrenocorticotropic hormone (ACTH) release in response to acute restraint stress, especially in females. Notably, both male and female GPER\(/-\) rats showed increased anxiety-like behaviors and deficits in learning and memory \(^{76}\). Taken together, these results reveal a regulation of the HPA axis and response to stress by GPER.

Depending on the dose and the timing of administration of G-1, anxiety-like behavior in rodents subjected to the elevated plus-maze and the open field test may either increase or decrease \(^{77–79}\). In human studies, Findikli and colleagues (2017) investigated GPER serum levels as a possible biomarker for major depressive disorder (MDD). They found significantly higher levels of GPER and anxiety symptoms among drug-naïve MDD patients, as well as a positive correlation between GPER1 levels and depression scores \(^{80}\). Although preliminary, this study suggests that GPER could have diagnostic value in human psychiatric disorders.
GPER in the Regulation of Cellular Mechanisms Relevant to Schizophrenia

GPER is present in microglial cells, neurons and astrocytes. It is broadly distributed in the brain, and especially highly expressed in the hypothalamus, hippocampus, substantia nigra, medulla oblongata, and pituitary, of laboratory animals. No significant differences in GPER expression have been observed between male and female rats, but functional assays have not been performed. The GPER expression pattern in the rat brain appears to be similar to that of GPER mRNA in the human brain. In humans, GPER expression is higher in the hippocampus and hypothalamic nuclei (supraoptic, paraventricular, arcuate, and suprachiasmatic nuclei) and less abundant in the cortex and caudate nucleus.

A critical cellular function of GPER that is relevant to schizophrenia is the regulation of intracellular Ca\(^{2+}\) stores. The Ca\(^{2+}\) signaling pathway plays a central role in regulating neuronal excitability, information processing, and cognition. In microglial cells, intracellular Ca\(^{2+}\) signaling is important for ramification, de-ramification, migration, phagocytosis, and the release of cytokines, nitric oxide (NO), and brain-derived neurotrophic factor (BDNF). Notably, BDNF induces a sustained intracellular Ca\(^{2+}\) elevation by upregulating canonical transient receptor potential 3 (TRPC3) channels in rodent microglia. TRPC3 may be important not only for BDNF anti-inflammatory effects but also for the synaptogenesis that is modulated by microglial phagocytic activity in the brain. Ca\(^{2+}\) in microglial cells has putative inflammatory effects that need further elucidation.

After binding to GPER, estrogen induces the transduction of signaling cascades that ultimately mobilize calcium stores. The GPER regulation of intracellular Ca\(^{2+}\) seems to depend on epidermal growth factor receptor (EGFR), which is activated by mitogen-activated protein (MAP) kinases and the extracellular-signal-regulated kinase (ERK) pathway. GPER also causes the activation of phospholipase C (PLC) and enhances inositol 1,4,5-trisphosphate production, which mobilizes intracellular Ca\(^{2+}\), leading to its release from the...
endoplasmic reticulum. There is some evidence that GPER controls Ca\textsuperscript{2+} signaling in response to estrogens in neural cells. GPER is highly expressed in spinal neurons, and its activation increases cytosolic Ca\textsuperscript{2+} and neuronal firing rates. Another target for GPER effects is the voltage-gated calcium channel (CaV) subunit α1D (Cav1.3). When estradiol binds to GPER, it activates the ERK pathway and increases Cav1.3 channels phosphorylation, which leads to a subsequent Ca\textsuperscript{2+} influx and may explain the pro-survival effects of estradiol. Of note, in a Ca\textsuperscript{2+}-free medium, G-1 (GPER agonist) elevates intracellular Ca\textsuperscript{2+} much less than in a Ca\textsuperscript{2+}-containing medium, which indicates that the activation of GPER mobilizes both extracellular and intracellular Ca\textsuperscript{2+} stores. In animals, the isoforms Cav1.2 and Cav1.3 are involved in hippocampus-dependent learning and memory, cognitive functions that require proper hippocampal neurogenesis and are impaired in schizophrenia.

In neurons, the GPER agonist G1 activates adenylyl cyclase with the consequent activation of protein kinase A (PKA), in a dose-dependent manner, activating the transcription factor cAMP response element-binding protein (CREB) and culminating with the transcription of several pro-survival and neurotrophic gene products, such as BDNF and Bcl-2. Also in neurons, GPER activation usually induces pro-survival pathways, such as phosphatidylinositol 3-kinase (PI3K)/Akt and ERK, and attenuates pro-apoptotic pathways. In the brain, the PI3K pathway regulates synaptic formation and plasticity. Its disruption leads to synapse dysfunction and pathological behaviors and is, thus, implicated in the pathogenesis of schizophrenia.

In addition, GPER seems to control microglial reactivity. Microglia are the immune CNS cells responsible for orchestrating the pro-inflammatory protective response against pathogens and injury. Aberrant microglial reactivity has been shown in the progression of several neuropsychiatric disorders. GPER attenuates the pro-inflammatory microglial...
phenotype by decreasing its phagocytic activity, NOS expression, and NO release. The anti-inflammatory and microglia-suppressing effects of estradiol in a rat model of stroke have been shown to depend on GPER activation. The GPER agonist G-1 acts in a similar way. This molecule attenuates microglial reactivity to bacterial endotoxin, lipopolysaccharide (LPS), as indicated by a reduction of mRNA and in levels of the pro-inflammatory cytokines, TNFα and IL-1β.

A relevant pro-inflammatory mechanism is the activation of the nuclear factor kappa B (NF-kB). It has been demonstrated that the anti-inflammatory effect of estradiol is mediated by ERα activation, which leads to the blockade of NF-kB activation and of its translocation to the nucleus. Although ERα has been held responsible for the main anti-inflammatory effects of estradiol on microglia, a recent study showed that GPER agonist G-1 also inhibits NF-kB nuclear migration and decreases the expression of the NLRP3-ASC-caspase 1 inflammasome and IL-1β activation. In line with this evidence, G1 was shown to increase the phosphorylation levels of CREB and enhance IL-1 receptor antagonism in rat hippocampus. By mechanisms dependent on NF-kB inhibition by GPER, this receptor regulates the activation of matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9. MMP-2 is detected in brain structures such as astroglia and some pyramidal neurons in the cortex and Purkinje cells in the cerebellum. MMP-9 is expressed in the hippocampus, cerebellum, and cortex, predominantly in neurons. Levels of MMP-2 and MMP-9 being significantly elevated following ischemia, brain injury, and kainate treatment, implies a role for MMP-2 and MMP-9 in the remodeling of neural circuits in response to neural activity and brain damages. MMP-9 is mainly involved in several key neurodevelopmental processes that are altered in schizophrenia, including maturation of calcium-binding protein parvalbumin inhibitory
neurons, the developmental formation of the specialized extracellular matrix structure perineuronal net, synaptic pruning, and myelination.

GPER anti-inflammatory and immunomodulatory effects seem to be markedly influenced by sex. After brain ischemia, the increase in GPER expression occurs only in male animals and exacerbates microglial reactivity and neuronal death. GPER agonist G-1 increases infarct volume in males poststroke but reduces it in ovariectomized females. By contrast, GPER activation has been shown to induce neuroprotective and anti-inflammatory effects in both male and female brains exposed to ischemia.

It deserves to be mentioned that GPER is also present in the placenta. In recent years, a link has been made between placental biology, early-life complications (preeclampsia and intrauterine growth retardation), and schizophrenia, even though the diagnosis of schizophrenia is not usually made until adult life. Placental GPER expression is reduced in women with preeclampsia as compared to women with uncomplicated pregnancies. Treatment with estradiol significantly increases the expression of GPER in HTR8/SVneo cells in both normal and hypoxia-reoxygenation conditions. Furthermore, decreased GPER expression has been detected in maternal serum and placenta and has been shown to associate with intrauterine growth retardation. Based on findings such as these, in future years, placental expression and/or maternal serum levels of GPER may emerge as biomarkers of schizophrenia risk.
GPER Agonists for Schizophrenia Drug Treatment

To date, there is no direct evidence that the pharmacological or genetic modulation of GPER would be beneficial in the treatment of schizophrenia, but this remains an interesting possibility. As previously mentioned, GPER modulates several intracellular mechanisms that are compromised in schizophrenia \textsuperscript{94,101}. There is compelling evidence of an aberrant pro-inflammatory microglial phenotype in the schizophrenia brain, which may explain the synaptic pruning disruption seen in adolescence and the progressive neurodegenerative changes that occur over time \textsuperscript{113,114}. Based on the intracellular mechanisms modulated by GPER and their relevance for schizophrenia \textsuperscript{115}, we speculate that GPER could become a useful pharmacological target for new immunomodulatory and potentially sex-specific treatment strategies.

Current antipsychotics are not effective for negative symptoms and cognitive impairments \textsuperscript{116}, but there is some evidence that estradiol’s adjunctive use can alleviate these symptoms \textsuperscript{117,118}. In preclinical models, estrogens influence social preferences and learning and memory of social stimuli \textsuperscript{71,119}. In female rats, estradiol treatment inhibits the disruption of prepulse inhibition in rodent models of schizophrenia by preventing dopamine D1/D2 receptor-mediated disruptions of sensorimotor gating \textsuperscript{120}. Although very few clinical studies using adjunctive estradiol in men with psychotic symptoms have been conducted, such studies are beginning to appear and to show positive effects \textsuperscript{121}. Hence, based on these studies \textsuperscript{122}, we speculate that a specific GPER agonist, devoid of sexual and other estrogen-associated adverse effects, may become an important therapeutic agent in schizophrenia. The following section discusses available GPER agonists and their possible benefits as safe therapeutic options in schizophrenia \textsuperscript{123}. We believe,
however, that, before performing clinical trials with GPER agonists, more evidence from preclinical models of schizophrenia on GPER expression and functions must be available and that GPER as a potential treatment target must be validated in blood and tissue samples from schizophrenia patients.

**Selective Estrogen Receptor Modulators (SERMs)**

SERMs, including tamoxifen and raloxifene, can act as estrogen agonists or antagonists, depending on the target tissue and the estrogen receptor involved. They affect transcriptional regulation by ERα and ERβ but lead to different effects in different tissues. Although SERMs are associated with health risks such as stroke, thromboembolism, and endometrial hyperplasia \(^{124–126}\), they are safer than standard estrogens. Raloxifene and tamoxifen are known to trigger neuroprotective mechanisms and reduce neural damage in different experimental models of neural trauma, brain inflammation, cognitive impairment, neurodegenerative and affective disorders. They represent promising therapeutic tools, capable of inducing profound brain structural remodeling following cerebral ischemia. \(^{20,68}\)

Raloxifene is the only SERM approved for long-term treatment, having been used in several clinical trials for both men and women with schizophrenia \(^{127}\). It acts as an estrogen agonist in the brain and an antagonist in mammary and uterine tissue \(^{128}\). A systematic review and meta-analysis of nine studies testing the effects of raloxifene in 561 women with schizophrenia spectrum disorders showed that raloxifene as an adjunct to antipsychotic medication is superior to placebo in improving total symptom severity, positive and negative symptoms \(^{129}\). This result holds true for trials in men and in postmenopausal women \(^{130}\). Adjunctive raloxifene treatment also improves attention/processing speed and memory of men and women with schizophrenia \(^{122,123,131}\).

Although there is substantial evidence that raloxifene significantly improves outcomes in patients with schizophrenia \(^{127,130,132}\), in some studies it only improves cognition
Some studies in severely decompensated postmenopausal women fail to show improvement. Results such as these suggest that symptom profile and severity must be considered when considering pharmacotherapy with SERMs. A recently published clinical trial showed that the ERβ agonist LY500307, used as an adjunct to antipsychotics, is selective, safe, and well-tolerated in patients with schizophrenia but fails to demonstrate any significant effects on brain targets: cognition, negative and total symptoms. Although dose and patient characteristics may explain the results, the data suggest that ERα activation may be necessary to yield positive results.

SERMs have been demonstrated to act as agonists of GPER and raloxifene has been shown to activate GPER in cells deficient for ERα. It is important to not that, at the doses used to treat schizophrenia, SERMs are relatively free of adverse events in either men and women. 17β-estradiol, SERMs, and selective estrogen receptor downregulators are all agonists of GPER, but, although estrogen and SERMs have shown promising effects in schizophrenia, it does not automatically mean that GPER will be equally effective. LNS8801 is an orally bioavailable small molecule that is a highly specific and potent agonist of GPER. A multi-center study to assess the safety, tolerability, pharmacokinetics, and preliminary anti-tumor activity of LNS8801 in patients with advanced cancer is currently underway. Other GPER agonists are under development as potential treatments for cancer. Over the next few years, therefore, we will know more about the safety and tolerability of GPER agonists in humans, paving the way for potential clinical trials in patients with schizophrenia.

Phytoestrogens

The neuroprotective effects of phytoestrogens, such as genistein and daidzein, have been recently discovered. Genistein is an estrogenic compounds naturally occurring in plants that shares structural features with 17β-estradiol. It exerts marked anti-inflammatory effects on microglial BV2 cells when
challenged with LPS. These effects are suppressed by pharmacological and genetic blockade of GPER, demonstrating the importance of GPER as an immunomodulatory target. Based on its similarity with 17β-estradiol and the results of preclinical studies, Genistein seems to be of potential clinical utility in managing psychiatric disorders. Quercetin is another phytoestrogen that has shown promising results in schizophrenia treatment. Like genistein, quercetin is a GPER agonist. A case report on two patients demonstrates that adjunctive therapy with quercetin can lead to clinical improvement in schizophrenia.

**Concluding Remarks**

GPER is an estrogen transmembrane receptor that mediates nongenomic estrogen actions. It is responsible for rapid estrogen anti-inflammatory and immunomodulatory effects. Based on research that demonstrates important advantages for pre-menopausal women relative to men on the course of schizophrenia, estrogens, SERMs, and phytoestrogens, which bind to GPER as well as to classical estrogen receptors, have been successfully used as adjuncts to antipsychotic medication.

The question arises whether GPER-targeted treatment can be effectively used in male and female schizophrenia patients. As mentioned above, the tissue distribution of ERα and ERβ varies across male and females; in rats, GPER mRNA expression is relatively stable across all tissues in both sexes. GPER's rapid, non-genomic actions play a central regulatory role in cardiovascular function, so that GPER agonists have been suggested as potential therapeutic agents in vascular and myocardial disease in both men and women. In rats, the GPER agonist G1 protects the heart from ischemia/reperfusion (IR) injury in both males and females. Nevertheless, things may be different in the brain where sex-differential effects are more likely. GPER is upregulated in male brain after stroke, and GPER agonist G-1 increases infarct volume in males poststroke but reduces it in...
ovariectomized females. By contrast, GPER activation seems to induce neuroprotective and anti-inflammatory effects in both male and female brains exposed to ischemia. Taken together, these results suggest that the sex-differential effects of GPER activation cannot be generalized across tissues. Nor is it clear how different GPER agonists and antagonists will act in men and women with schizophrenia.

Despite increasing interest in GPER, little is known about its potential as a therapeutic target in schizophrenia. This review suggests that it is worth investigating.
Authors Contributions

RC, CFM and ASM – performed the first selection of the articles
LLOS, AJMCF, and DSM – refined the selection of the articles and constructed the tables
DSM, DFL, CFM, ASM, and AJMCF – wrote the first draft
LLOS, MVS and DSM – organized the final version of the manuscript
Fig. 1. Graphic representation of 17β-estradiol (E$_2$) modulating CNS functions and the distribution of E$_2$ receptors in the brain and associated signaling pathways. In the figure, in the upper right corner, the 2D structure of estradiol (E$_2$) is represented as well as its main modulating functions in CNS. The numbers (blue heptagons) represent the brain regions where E$_2$ receptors are mainly expressed. Of note, 1: prefrontal cortex; 2: basal forebrain; 3: hypothalamus; 4: amygdala; 5: thalamus; 6: hippocampus; 7: raphe nucleus; 8: locus coeruleus; 9: posterior cingulate. In the lower-left corner, the E$_2$ receptors subtypes are represented: the nuclear ERα & β receptors, responsible for the main genomic effects of E$_2$ signaling, and the metabotropic GPER1 receptor, responsible for second-messenger rapid transduction of E$_2$ (non-genomic effects). Also, we built a 3D structural representation of human GPER1 protein based on the homology to the template 4n6h1.A (PDB ID), using SwissModel server. Abbreviations: E$_2$, 17β-estradiol; CNS: central nervous system; ER: estrogen nuclear receptor; GPER: G protein-coupled receptor.
Fig. 2. Pathways regulated by 17β-estradiol binding to GPER of relevance to schizophrenia neurobiology. Estradiol binding to GPER activates the ERK pathway and increases phosphorylation of Cav1.3 channels, which leads to subsequent Ca2+ influx, which may explain the pro-survival effects of estradiol. In animals, Cav1.3 channels are involved in hippocampus-dependent learning and memory, cognitive functions that require proper hippocampal neurogenesis, and are impaired in schizophrenia. In neurons, GPER activates adenylyl cyclase, with the consequent activation of protein kinase A (PKA), and the transcription factor cAMP response element-binding protein (CREB), leading to the transcription of several pro-survival and neurotrophic gene products, such as BDNF and Bcl-2. In neurons, GPER activation induces pro-survival pathways, such as phosphatidylinositol 3-kinase (PI3K)/Akt and ERK, and attenuates pro-apoptotic pathways. By mechanisms dependent on NF-kB inhibition by GPER, this receptor regulates the activation of matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9, which are dysregulated in neurodegenerative disorders, including schizophrenia. In the figure, dashed lines represent inhibition while continuous lines represent stimulation.
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