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Pregnane Xenobiotic Receptors and Membrane Progesterin Receptors: Role in Neurosteroid-Mediated Motivated Behaviors

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Abstract

Progesterogens have actions in the midbrain ventral tegmental area (VTA) to mediate motivated behaviours, such as those involved in reproductive processes, among female rodents. In the VTA, formation and actions of one progesterone, 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP; 3 α ,5 α -THP), are necessary and sufficient to facilitate sexual responding (measured by lordosis) of female rodents. Although 3 α ,5 α -THP can be produced following metabolism of ovarian progesterone, 3 α ,5 α -THP is also a neurosteroid produced *de novo* in brain regions, such as the VTA. There can be dynamic changes in 3 α ,5 α -THP production associated with behavioural experience, such as mating. Questions of interest are the sources and targets of 3 α ,5 α -THP. Regarding sources, pregnane Xenobiotic Receptor (PXR) may be a novel factor involved in 3 α ,5 α -THP metabolism in the VTA (as well as a direct target of 3 α ,5 α -THP). We have identified PXR in the midbrain of female rats, and manipulating PXR in this region reduces 3 α ,5 α -THP synthesis and alters lordosis as well as affective and social behaviours. Regarding targets, recent studies have focused on the role of membrane progesterin receptors (mPRs). We have analyzed expression of two of the common forms of these receptors (mPR α /paqr7 and mPR β /paqr8) in female rats. Expression of mPR α was observed in peripheral tissues and brain areas, including hypothalamus and midbrain. Expression of mPR β was only observed in brain tissues and was abundant in the midbrain and hypothalamus. To our knowledge, studies of these receptors in mammalian models have been limited to expression and regulation, instead of function. A question that was addressed was the functional effects of progesterogens via mPR α and mPR β in the midbrain of hormone-primed rats for lordosis. Studies to date suggest that mPR β may be an important target of progesterogens in the VTA for lordosis. Together, these studies demonstrate that PXR is involved in production of 3 α ,5 α -THP in the midbrain VTA. Moreover, mPRs may be a target for progesterogens' actions in the VTA for lordosis.

Keywords

progesterone; non-genomic; lordosis; neurosteroids; mating

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Conflicts of Interest

The authors have no conflicts of interest to report.

Introduction

The role of progesterone, and other progestogens (referring herein to progesterone and its neuroactive products, including 5 α -pregnan-3 α -ol-20-one; 3 α ,5 α -THP), beyond their pro-gestational effects are of interest. Progestogens are involved in facilitating successful mating before gestation, have organizing effects on the nervous system during gestation/perinatally and then can alter adult behaviours that ultimately are adaptive (reducing stress/anxiety, enhancing cognition and conferring protection to neural insults/aging; see reviews on these topics by: [1–5]). Of interest are the novel sources and targets of progestogens for such effects.

To be able to ask questions about the sources and targets of progestogens for effects on varied central nervous system functions, it is useful to focus on a behaviour that is reliant upon progestogens, and then extend this approach to other behaviours that are regulated by progestogens. As such, in our laboratory, we utilise mating behaviour of female rodents, with one measure being the lordosis response. Lordosis refers to the mating posture of female rodents to allow intromissions by the male. Lordosis is a quantifiable behaviour under hormonal control, and the brain circuitry underlying it is well-characterised [6–8]. Lordosis is observed under appropriate endocrine and environmental contexts, including progestogens having actions in midbrain and hypothalamic regions. By using such a behaviour that is dependent upon progestogens' actions in the midbrain, for example, we can investigate the requisite factors for formation as well as actions of progestogens in this region. These studies then inform subsequent investigations on how these same mechanisms may be conserved across species, brain regions and other behaviours (e.g. affect, affiliation, cognition, and neuroprotection).

From studies using this approach, it is clear that progesterone has actions in the midbrain ventral tegmental area (VTA) to mediate motivated behaviours, such as those involved in reproductive processes, among female rodents. In the VTA, formation and actions of the progestogen, 3 α ,5 α -THP (a.k.a. allopregnanolone), are necessary and sufficient to facilitate lordosis (reviewed recently in [9]). Although 3 α ,5 α -THP can be produced following metabolism of ovarian progesterone, 3 α ,5 α -THP is also a neurosteroid produced *de novo* in brain regions, such as the VTA. Because actions of 3 α ,5 α -THP are necessary and sufficient in the VTA for lordosis, we have been utilizing this behaviour as a bioassay to ask questions about factors involved as sources and targets of progestogens, which will be discussed throughout this review. Indeed, these studies addressing mechanistic questions about progestogens in the VTA using lordosis as the *in vivo* assay are then extended to other areas of interest regarding progestogens' effects across the lifespan.

Another intriguing finding related to 3 α ,5 α -THP's vital role in reproductive behaviours is that there can be dynamic changes in 3 α ,5 α -THP production associated with behavioural experiences, such as mating. For example, we have demonstrated that midbrain 3 α ,5 α -THP levels are highest following paced mating (where female control the timing of male contacts' [10,11]). In addition to such a response from a social challenge like mating, previous work by Purdy and colleagues demonstrating rapid and robust changes in 3 α ,5 α -THP synthesis following acute environmental and physical stressors (footshock, swim stress; [12]). Moreover, chronic stressors during gestation (e.g. restraint stress, immune challenges, environmental disruptions of dams) or adulthood (social isolation) reduce 3 α ,5 α -THP levels. Additionally, reducing 3 α ,5 α -THP synthesis in the brain is associated with greater stress responding [13–18] As such, one notion is that 3 α ,5 α -THP is critical for homeostatic regulation.

This review will focus on the identifying novel sources and targets of $3\alpha,5\alpha$ -THP in the midbrain VTA. First, novel sources of $3\alpha,5\alpha$ -THP will be a focus. Previous work demonstrating that manipulating enzymes for $3\alpha,5\alpha$ -THP formation in the midbrain attenuates lordosis will be discussed. How these enzymes may be downstream of a novel factor, the pregnane xenobiotic receptor (PXR), and its role as a key regulatory factor for behavioural-induced $3\alpha,5\alpha$ -THP formation in the midbrain will be addressed. Second, novel targets of $3\alpha,5\alpha$ -THP will be a focus. Studies establishing that $3\alpha,5\alpha$ -THP has rapid effects that do not involve nuclear progesterin receptors (PRs) in the VTA will be discussed. Then, the role of membrane progesterin receptors (mPRs) as targets of progestogens in the midbrain for lordosis will be addressed. Studies are described that support PXR as a novel factor in production of, and mPRs as novel targets of, $3\alpha,5\alpha$ -THP, using the lordosis model, in its role as a regulator of homeostatic responses, related to stress, motivated behaviours and plasticity (Figure 1).

Sources of $3\alpha,5\alpha$ -THP

Formation of $3\alpha,5\alpha$ -THP can occur following metabolism from progesterone from peripheral glands (e.g. ovaries, adrenals, placenta) or its *de novo* synthesis. Regarding metabolism, progesterone, secreted from peripheral glands, travels in circulation to the brain (as well as many other target organs) and then can be metabolised by enzymes to other neuroactive metabolites. A pathway to form $3\alpha,5\alpha$ -THP from metabolism of progesterone involves sequential actions of 5α -reductase (an irreversible action that forms dihydroprogesterone) and then 3α -hydroxysteroid dehydrogenase (3α -HSD). Oestradiol can enhance progestogen metabolism, and studies in our laboratory suggest that the beta form of the estrogen receptor may be involved for such effects (reviewed in [19]). However, these progestogens can also be formed in the brain itself, independent of these peripheral sources [20–27]. The biosynthetic pathway for neurosteroid production involves many recognized factors, including the 18kDA translocator protein (TSPO, a.k.a. peripheral-type benzodiazepine receptor), the steroidogenic acute regulatory (StAR) protein, cytochrome P450-dependent C27 side chain cleavage enzymes (P450_{scc}), 3β -hydroxysteroid dehydrogenase (3β -HSD), 5α -reductase, and 3α -HSD. TSPO and StAR have actions to transport cholesterol (a requisite precursor for all steroids) into the mitochondria. In the mitochondria, cholesterol is oxidised by P450_{scc} to form, which is then metabolised by 3β -HSD to progesterone. Progesterone can then be metabolised to form $3\alpha,5\alpha$ -THP, via actions of 5α -reductase and 3α -HSD. Thus, $3\alpha,5\alpha$ -THP can be formed following conversion of peripheral and central sources of progesterone.

Reducing activity at any of these rate-limiting steps for biosynthesis and/or metabolism to $3\alpha,5\alpha$ -THP in the midbrain VTA attenuates lordosis of rodents. First, these enzymes are expressed in the brain, with high levels reported in the midbrain and corticolimbic regions of rats [28–32]. Using RT-PCR and western blotting, we have identified mRNA and protein expression of StAR, P450_{scc}, 3β -HSD, 5α -reductase, and 3α -HSD in the midbrain of pro-oestrous rats (those that are naturally-receptive with high progestogen levels; [33]). Second, infusions of antagonists to these targets to the VTA of receptive rats reduce lordosis responding ([5,9,34–36], See Figure 2). Third, genetic manipulations support these effects of pharmacological manipulations of these factors for lordosis. For example, mice that are deficient in 5α -reductase have lower lordosis as well as midbrain $3\alpha,5\alpha$ -THP levels when primed with oestradiol and progesterone, compared to wildtype mice [37]. Yet, 5α -reductase knockout mice have increased lordosis when administered $3\alpha,5\alpha$ -THP [37], suggesting that the deficit in lordosis is related to formation of $3\alpha,5\alpha$ -THP, not responses to $3\alpha,5\alpha$ -THP. Fourth, studies investigating age-related changes in reproductive behaviours support the notion that alterations in formation of $3\alpha,5\alpha$ -THP in the brain modifies reproductive responses. Among middle-aged rats, there is variability in the timing of reproductive

senescence (defined by acyclicity, low fertility, and low fecundity) and mating behaviours, which may be related to capacity to metabolise progesterone and dihydroprogesterone in the midbrain as well as reward pathway targets, such as the diencephalon [38]. Fifth, we have observed the same pattern of effects for such central manipulations of these factors required for 3 α ,5 α -THP synthesis in gonadally-intact, ovariectomised, or ovariectomised and adrenalectomised rats, suggesting that peripheral sources of progesterone were not necessary for these effects [5]. Together, these studies demonstrate the importance of 3 α ,5 α -THP in the midbrain for lordosis; however, another question is the role of mating for biosynthesis of 3 α ,5 α -THP in the brain.

Mating induces 3 α ,5 α -THP formation in the brain. Levels of 3 α ,5 α -THP are increased in the midbrain VTA following mating [5,9–11,30,31]. Other reproductively-relevant behaviours, such as exploration (in an open field), anxiolysis (in an elevated plus maze), or social interaction with another female do not produce the same increases in 3 α ,5 α -THP in the midbrain as does mating among pro-oestrous rats [10,11]. In addition to being specific to mating, this behaviour-induced 3 α ,5 α -THP synthesis is most robust following a semi-naturalistic mating paradigm utilized in the lab, called paced mating, compared to a standard mating paradigm. With paced mating compared to standard mating, the testing chamber is larger and divided so that females can control the timing (i.e. “pace”) of their sexual contacts with males. The pacing task is then more akin to a naturalistic mating scenario among rats, is spontaneously observed among naïve females, and enhances reproductive success (increases fertility and fecundity; [40]). Among females that do not spontaneously pace their contacts, despite being exposed to and mating with a male in a pacing chamber, there are lower levels of 3 α ,5 α -THP in the midbrain following testing, compared to that observed among females that do spontaneously pace in the task [41]. As well, there are greater increases in 3 α ,5 α -THP in the midbrain following paced compared to standard mating [5,9–11]. Notably, behaviour-induced biosynthesis of 3 α ,5 α -THP does not only occur in the midbrain. Following paced mating, there are rapid enhancements in the levels of 3 α ,5 α -THP in the hypothalamus, striatum/diencephalon, hippocampus and cortex [41], further support of a role of 3 α ,5 α -THP for behaviours beyond reproduction. As well, behavioural-induced biosynthesis of 3 α ,5 α -THP occurs rapidly, and in the absence of peripheral sources of progestogens, such as the ovaries, adrenals, and placenta, suggesting that the brain is the source for mating-induced 3 α ,5 α -THP formation. A question of continued investigations is the upstream factors that may be driving this response.

Role of pregnane xenobiotic receptor

Current investigations are aimed at understanding the role of PXR as a novel factor for 3 α ,5 α -THP's synthesis in the midbrain VTA. A well-known function of PXR is to detect both exogenous and endogenous toxins and up-regulates gene transcription for proteins that metabolize and/or dispose of these. A focus heretofore has been on these actions of PXR in the liver and other secretory organs (e.g. intestines and kidneys) as a “master regulator” in these tissues [42–45], but more recent work supports the importance of PXR for clearance and metabolism in the nervous system (described below). Activated PXR regulates gene transcription in a ligand-dependent fashion, which promotes the production of a wide array of proteins, including CYP enzymes, which metabolize many drugs and hormones [45–46]. Indeed, 3 α ,5 α -THP is a ligand of PXR (albeit, the specificity of this response is not entirely clear [45–46]), further suggesting interaction of PXR and 3 α ,5 α -THP in regulating homeostatic processes. Current studies are investigating the role of PXR for cholesterol metabolism/clearance and induction of CYP enzymes important for neurosteroidogenesis.

The evidence in support of a potential role of PXR in central nervous system function, including neuroendocrinology, is growing. First, PXR is expressed in the brain. The

traditional thinking was that PXR was predominantly a liver factor. However, there has been identification of PXR in the central nervous system of several species, such as rodents, rabbits, pigs, and [9,33,46–50]. Of particular importance to behavioural-induced $3\alpha,5\alpha$ -THP synthesis, PXR is expressed in the midbrain VTA. Studies using microarray, RT-PCR and qPCR, western blotting and enzyme-linked immunosorbance assays have confirmed the presence of PXR gene, RNA, and protein in the midbrain of mated female rats [9,10,33,51]. PXR expression in the midbrain may be sensitive to hormonal state as pro-oestrous rats have higher levels than do dioestrous rats or male rats [9,10,33,51]. Second, activation of PXR with ligands, such as progestogens $3\alpha,5\alpha$ -THP enhance lordosis responding of dioestrous or ovariectomised, oestradiol-primed rats [52–53]. Given that PXR is expressed in brain and body and can be activated by many environmental and pharmacological agents to alter many different CYP enzymes that are involved in steroid metabolism and clearance of many drugs, it was important to begin investigating the specificity of these effects of PXR manipulations. Third, studies using knock down of expression of PXR in the midbrain VTA with infusions of antisense oligodeoxynucleotides (AS-ODN) targeted to PXR have revealed some of its functional effects. Among cycling rats, PXR AS-ODNs reduced midbrain PXR expression and $3\alpha,5\alpha$ -THP levels and attenuated lordosis responding, proceptivity and aggression/rejection behaviours towards the males, of pro-oestrous rats [9,10,33,51]. There were no effects of AS-ODN infusions to dioestrous rats, which have low progestogens and reduced capacity for mating-induced increases in $3\alpha,5\alpha$ -THP levels. Over the estrous cycle, there are sequential increases in oestradiol followed by elevations in progestogen levels during pro-oestrus. A question is then PXR manipulations are oestradiol- and/or progestogen-dependent. Additionally, oestradiol enhances 5α -reductase activity, consequently increasing brain $3\alpha,5\alpha$ -THP, and is required for $3\alpha,5\alpha$ -THP to facilitate lordosis of ovariectomised female rats [34]. As such, whether there are distinct effects of oestradiol to involve PXR was investigated. Among ovariectomised rats primed with oestradiol (but not vehicle alone) and administered $3\alpha,5\alpha$ -THP and PXR AS-ODNs to the VTA, there was a reduced capacity to respond to $3\alpha,5\alpha$ -THP infusions or behavioural-induced $3\alpha,5\alpha$ -THP production in the midbrain [54]. These data show that replacement of $3\alpha,5\alpha$ -THP following knock down of PXR in the VTA does not reverse effects of PXR knock down for lordosis, suggesting binding of $3\alpha,5\alpha$ -THP to PXR may be important for these effects on lordosis and mating-induced $3\alpha,5\alpha$ -THP. Fourth, studies are ongoing to characterize the behaviour and (neuro)steroid responsiveness of commercially-available animal models that are genetic knockouts of PXR (mice from Taconic Inc, and rats from S.A.G.E. Labs). Moreover, how these PXR manipulations may alter expression and/or activity of downstream factors important for neurosteroidogenesis are of ongoing interest. Together, these data suggest that PXR is a homeostatic regulator for behavioural-induced $3\alpha,5\alpha$ -THP formation.

Potential role of liver X receptor

Another potential target of interest as a novel factor involved in production of $3\alpha,5\alpha$ -THP is the liver X receptor, a relative of PXR as a member of the nuclear receptor superfamily. $3\alpha,5\alpha$ -THP can be produced, and secreted, by both glia and neurones, independent of peripheral secretion; indeed, it has been suggested that LXR and PXR may have respective roles in glia and neurones [1]. Although both of these receptors have been traditionally considered in their roles as liver factors, evidence is mounting regarding their expression and functional effects in the central nervous system. The studies investigating expression and functions of PXR in the brain are described in the previous section. In the case of LXR, there have been recent reports of its role in neurogenesis in the developing mouse midbrain [55] as well as an anxiety phenotype in the female LXR knockout mouse [56]. Indeed, a consideration is the role of these receptors for cholesterol metabolism and clearance, which has clinical relevance for development, aging and neurodegenerative disorders (e.g.

Alzheimer's disease, multiple sclerosis, diabetes neuropathy; Niemann-Pick disease; 46, 57–62). Indeed, a role of $3\alpha,5\alpha$ -THP has likewise been implicated in these disorders [1,3,5,63,64]. Of interest to us is how PXR and LXR may act synergistically for efficient cholesterol metabolism and mating-induced $3\alpha,5\alpha$ -THP, and how these effects may, ultimately, be neuroprotective or facilitate plasticity.

Targets of $3\alpha,5\alpha$ -THP

Following its formation in the midbrain VTA, a question is by what receptor mechanism may $3\alpha,5\alpha$ -THP be having its functional effects. Traditional notions about how steroids have effects are those that involve binding to their intracellular cognate steroid receptors. Cognate, nuclear progesterin receptors (nPRs) were first characterized ~40 years ago [65]. As with the traditional actions of other steroid receptors, nPRs bind ligands and act as transcription factors to evoke expression of mRNAs leading to changes in protein synthesis, and, thereby, biological actions. Both progesterone and dihydroprogesterone bind with a high affinity for intracellular progesterin receptors, which are located in reproductive tissues and brain areas, such as the hypothalamus [66].

Studies investigating the mechanisms of action of $3\alpha,5\alpha$ -THP suggest that it does not require actions at nPRs for its functional effects on lordosis. This idea has been supported by several studies focused on investigating whether $3\alpha,5\alpha$ -THP in the midbrain VTA meets criteria for steroid receptor actions in this region: 1) receptors are expressed; 2) activation of receptors increase behavioural response; 3) blockade of receptors attenuate behavioural response; 4) genetic knockouts of receptors show an attenuated behavioural response; 5) timing suggests effects are too rapid for new protein synthesis; 6) effects are observed when relegated to the cell membrane. First, nPRs are low in the VTA, and there is little evidence of oestradiol-induced nPRs in this region [30,31,66]. Second, activating nPRs with nPR agonists directed at the VTA do not enhance lordosis of ovariectomised rodents [7,30,31]. Third, conversely, nPR antagonists do not alter progesterone-facilitated lordosis [67–69]. Fourth, there are similar effects of progestogens to increase lordosis of nPR knockout mice and their wildtype counterparts [70]. Fifth, progestogens to the VTA have rapid effects to increase neuronal firing here as well as lordosis (all in less than 5 minutes, which is considered outside of the timeframe for mRNA translation, transcription and new protein synthesis; [71]). Sixth, unbound progestogens permeate cell membranes with ease, but if they are conjugated to a large protein, like bovine serum albumin, passage into the cell is restricted; yet, conjugated progestogens to the VTA enhance lordosis of ovariectomised rodents [72,73]. Together, these studies demonstrated that $3\alpha,5\alpha$ -THP has rapid, membrane-relegated effects that do not require nPRs in the VTA for lordosis of female rodents.

$3\alpha,5\alpha$ -THP, like other neurosteroids, has actions through ion channel-associated membrane receptors for rapid functional changes, and there are several of these targets in the VTA to consider in their role for progesterone-facilitated lordosis. There is a long history of investigating the role of $3\alpha,5\alpha$ -THP through γ -aminobutyric acid type A ($GABA_A$) receptors. $3\alpha,5\alpha$ -THP, even compared to $GABA_A$ ergic anxiolytics (benzodiazepines and barbiturates), has higher efficacy at $GABA_A$ receptors, and directly increases chloride channel currents when in very low (nanomolar) concentrations [74–78]. The effects of $3\alpha,5\alpha$ -THP as a positive modulator $GABA_A$ receptors have been well-characterized in the VTA [7,30,31] and elsewhere in the brain [79–81]. Blocking $GABA$ receptors in the VTA attenuates lordosis of female rodents [7,30,31,82]. The midbrain VTA is a well-described part of the dopaminergic reward pathway, and progestogens can alter density of dopamine type 1 receptors as well as dopamine release in the midbrain [30,31]. We have demonstrated that blocking dopamine type 1, but not type 2, receptors in this region reduces progesterone-facilitated lordosis of female rodents; and lordosis can be enhanced with

dopamine type 1 agonists infused to the VTA [7,83,84]. Thus, actions via GABA_A and dopamine type 1 receptors in the VTA may be required for 3 α ,5 α -THP lordosis of female rodents.

Regarding the circuitry in the VTA, a suggestion is that there is an interaction between these effects of 3 α ,5 α -THP via GABA_A, dopamine and glutamate targets (as well as their downstream signal transduction pathways; [7]). In the VTA, there are dopamine type 1 receptor-expressing GABAergic terminals that synapse on dopamine cell bodies that express GABA_A and NMDA receptors [7,85,86]. There are also GABAergic interneurons in the midbrain VTA that provide input to dopamine-containing cell bodies and therefore enhance dopamine release when activated by GABA [87]. As well, activation of dopamine type 1 receptors on GABAergic afferents in the VTA enhances GABA release [88], and we have demonstrated that blocking GABA_A receptors in the VTA attenuates progesterone-facilitated lordosis and effects of dopamine type 1 agonists to enhance lordosis [89]. Furthermore, 3 α ,5 α -THP is a negative modulator of glutamate, reducing its excitatory effects [90], and 3 α ,5 α -THP increases levels of glutamate in the midbrain [30]. NMDAR agonists to the VTA enhance lordosis [91]. Thus, some of the membrane neurotransmitter targets in the VTA that are important for 3 α ,5 α -THP-facilitated lordosis are GABA, dopamine and glutamate.

A series of experiments investigated the downstream targets of 3 α ,5 α -THP via these neurotransmitter receptors in the midbrain VTA for lordosis by determining the requisite roles of G-proteins, adenylyl cyclase, phospholipase C, and protein kinases. These studies demonstrated in ovariectomised hormone-primed rats and hamsters that pharmacologically blocking G-proteins, adenylyl cyclase, phospholipase C, and protein kinases A or C in the midbrain VTA, attenuated progesterone-facilitated lordosis, and enhancements by GABA_A and dopamine type 1 receptor agonists (reviewed in [7]). An additional question is whether 3 α ,5 α -THP may be having actions via other G-protein coupled receptor targets in the VTA for these effects on lordosis. One such target of interest is membrane progesterin receptors (mPRs).

Role of membrane progesterin receptors

mPRs were characterized over a decade ago in aquatic species [92]. These receptors are members of the progesterin and adiponectin (PAQR) family, which contains 4 adiponectin receptor like (class I receptors), 5 unique mPR members (α ~ ϵ , class II receptors) and two hemolysin receptor like receptors [93–95]. The members of class II PAQR family, which consist of mPR α ~ ϵ , exist only in vertebrates, and members of the other two classes are present both in vertebrates and non-vertebrates, including yeast and bacteria [93,95]. The mPRs are conserved across a broad range of many vertebrates, from fish to humans [92,93,95], and specifically bind progesterones in these species: e.g. fish (goldfish, seatrout, and zebrafish), frog, and mammals (cattle, rat, mouse, human) [93,96–98]. Thus, mPRs are conserved across species.

It can be argued that mPRs are most well-known for their roles in reproductive processes based upon their expression patterns. A focus herein will be on the most well-studied of the mPRs, mPR α and mPR β (paqr7 and paqr8, respectively). mPR α was first characterized in spotted seatrout, and is expressed abundantly in reproductive (testes, ovary, uterus), kidneys, and brain and spinal cord among vertebrates, including fish, mice and human [92,99–101]. Functions of mPR α for reproductive processes have been described relating to rapid progesterone signaling in maturation of fish oocytes [92, 97, 99]; sperm motility in fish [98], progesterone-signaling in human myometrial cells, breast cancer cells and lymphocytes [95, 102–104], activity in rat and sheep reproductive tissues [105,106], and rapid gonadotropin releasing hormone secretion among mice [107]. mPR β is also proposed to be involved in

reproductive functions, including the controlled beating of cilia of the fallopian tubes of mice and humans [108]. Thus, this comparative literature supports a role of mPR α and mPR β in reproduction across species. Of continued interest is the role of these receptors as targets of progestogens, such as 3 α ,5 α -THP, for behavioural effects.

Beyond effects in peripheral reproductive organs, evidence is growing for the role of mPRs in the central nervous system. Two of the receptor subtypes that have received the most interest are mPR α and mPR β . We have demonstrated with RT-PCR that there are differences in expression of mPR α and mPR β in the body (spleen, heart, lung, kidney, liver, intestines) and brains (prefrontal cortex, hippocampus, amygdala, hypothalamus, midbrain) of pro-oestrous rats and mice. Among rats, mPR α is expressed in the body and brain (with high levels in the midbrain; [109]). That there was great abundance of mPR α found in both central and peripheral tissues of rats could indicate a coordinated up-regulation of the receptor in pro-oestrus. There was more specific expression of mPR β in the brain versus the body, with high levels in the midbrain [109]. Other studies have confirmed such differences, such that mPR β > mPR α protein expression in the rat brain, specifically the midbrain and hypothalamus [110,111]. In addition to this evidence of region-specificity, there are hormonal influences for expression of these mPRs. mPR α and mPR β are increased during pro-oestrus, compared to diestrus, of rats in the mediobasal hypothalamus [112]. Expression of mPR β is higher than mPR α in the midbrain, hippocampus, lateral and medial septum, and thalamus of ovariectomised, hormone-primed rats [110–112]. Indeed, there seems to be regulation of mPR expression in rats by oestradiol in these brain regions involved in reproductive behaviours [109]. Recent studies have begun characterizing the expression of mPRs among mice. Among pro-oestrous mice, there was abundant expression of both mPR α and mPR β in brain (including the midbrain), but lower expression in peripheral tissues assessed compared to that observed in the brain [113]. Thus, these data suggest that the midbrain may be one brain regions containing mPRs as a target of progestogens among rats and mice.

The functional effects of mPR α and mPR β among rats and mice are of interest. Rapid change in lordosis of rodents is one of the best-studied *in vivo* models for novel, rapid actions of progestogens. As such, to investigate this question, infusions of AS-ODNs targeted against mPR α and/or mPR β were infused intracerebroventricularly (ICV) or to the VTA of ovariectomised, hormone-primed rats and mice. Among rats, administration of mPR α AS-ODNs ICV reduced lordosis and expression of mPR α in the hypothalamus and VTA. Infusions to the VTA of AS-ODNs targeted against mPR α and/or mPR β reduced respective expression of these receptor subtypes in the VTA. Moreover, mPR β or mPR α + mPR β , but not mPR α , AS-ODNs significantly reduced lordosis quotients, lordosis ratings, and proceptivity quotients, and significantly increased aggression/rejection behaviours towards males compared to control infusions. This pattern of reproductive behaviour was only observed among rats that were oestradiol and progesterone-primed, not those primed with oestradiol alone, suggesting the progestogen-dependency of these effects. These data support the role of mPR β as a target of progestogens in the midbrain VTA for sexual receptivity of rats [109]. Among mice, comparisons were made for manipulations of mPR α and β among those on a C57, C57 \times 129 wildtypes, or nPR knockout background for reproductive responses following infusions of AS-ODNs ICV or to the VTA. Among mice, lordosis was reduced with mPR α , mPR β , or mPR α +mPR β AS-ODNs infused ICV [61]. When infused directly to the VTA, only mPR β or mPR α +mPR β AS-ODNs reduced lordosis. These effects were observed in C57 or C57 \times 129 wildtypes, but not in nPRKO mice [113]. Together, these data support a functional role of mPR β in the midbrain VTA as a likely target of progestogens for rapid facilitation of lordosis (See Figure 3).

Conclusion

The midbrain VTA is a well-described part of the dopaminergic reward pathway as well as important for naturally-motivating behaviours, such as mating (Frye, 2009). Studies discussed herein describe the role of novel factor involved in production of (e.g. PXR), and targets for (e.g. mPRs), the neurosteroid, 3 α ,5 α -THP, to have actions in the VTA for reproductive behaviours and other social challenges. Progestogens, such as 3 α ,5 α -THP, are necessary for the full complement of behaviours important for successful mating (i.e. social contacts, pacing of such contacts, lordosis, suppression of anxiety and aggression in favor of pro-affiliative behaviours). Moreover, engaging in paced mating enhances 3 α ,5 α -THP in the brain rapidly and without reliance on peripheral sources of prohormones, such as progesterone. If part of the progestogen metabolic or biosynthetic pathway is blocked in the midbrain, including PXR, we attenuate 3 α ,5 α -THP facilitated lordosis and mating induced biosynthesis of 3 α ,5 α -THP. We have recently observed that knocking down PXR in the VTA reduces expression of brain derived neurotrophic factor in the hippocampus [59], substantiating further studies on plasticity in this circuitry for social challenges, such as mating. Neurosteroids, such as 3 α ,5 α -THP, are endogenous neuromodulators, synthesized *de novo* in brain, that have actions at membrane targets, such as neurotransmitters as well as G-protein coupled receptors, such as mPR β in the midbrain VTA. Together, these studies demonstrate that PXR is a novel target involved in production of 3 α ,5 α -THP in the midbrain VTA, acting as a homeostatic regulator. Moreover, mPRs may be a novel target for progestogens' actions in the VTA for lordosis. How these targets involve behavioural and neural plasticity is of continued interest.

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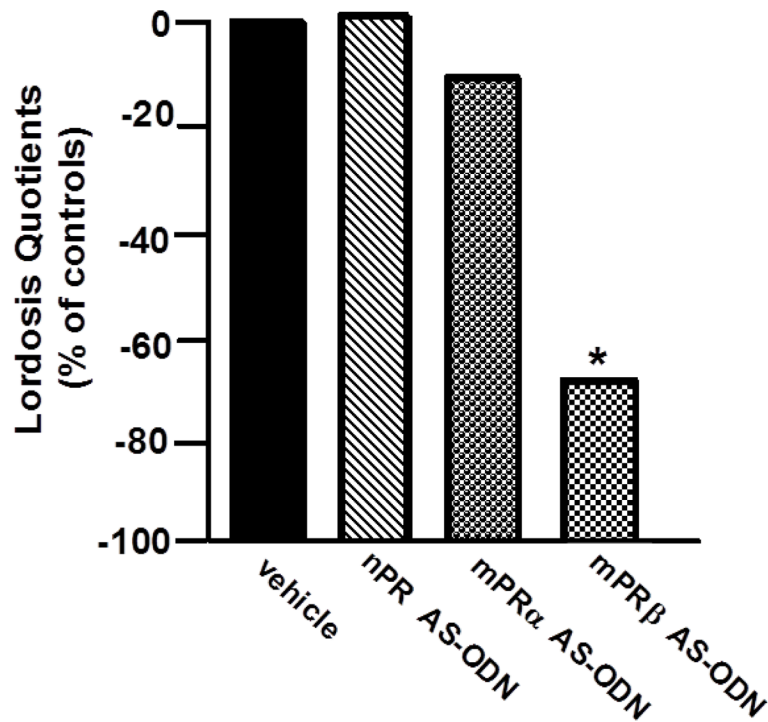


Figure 1.

Proposed relationship between pregnane xenobiotic receptor (PXR), $3\alpha,5\alpha$ -THP, and target substrates that may be involved in biosynthesis of $3\alpha,5\alpha$ -THP in the midbrain ventral tegmental area (VTA) with mating, including oestradiol role via oestrogen receptor beta ($ER\beta$). Moreover, $3\alpha,5\alpha$ -THP is a ligand of PXR and, thus, may activate PXR to underlie behaviour (mating) induced neurosteroidogenesis. $3\alpha,5\alpha$ -THP has novel membrane targets including GABAA, dopamine type 1 (D1), NMDA and membrane progesterin receptors (mPRs) that are important for actions of $3\alpha,5\alpha$ -THP in the VTA for processes underlying homeostasis, such as stress responding, motivated behaviors and plasticity (e.g. brain-derived neurotrophic factor; BDNF).

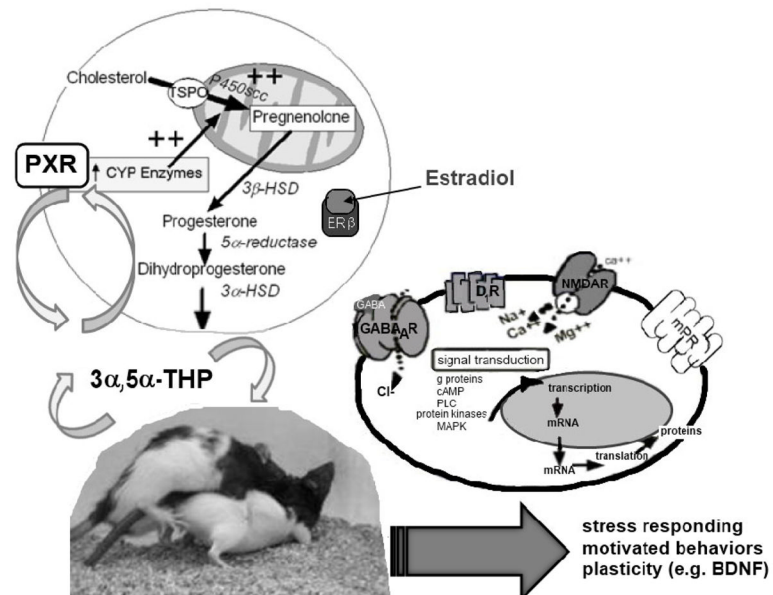


Figure 2. Intra-ventral tegmental area (VTA) infusions of positive modulators enhanced lordosis (bars) and increased 3α,5α-THP(circles). Infusions of inhibitors blocked increases of 3α,5α-THP levels in the VTA and decreased lordosis quotient. * indicates significant difference from vehicle-infused receptive controls, $p < 0.05$.

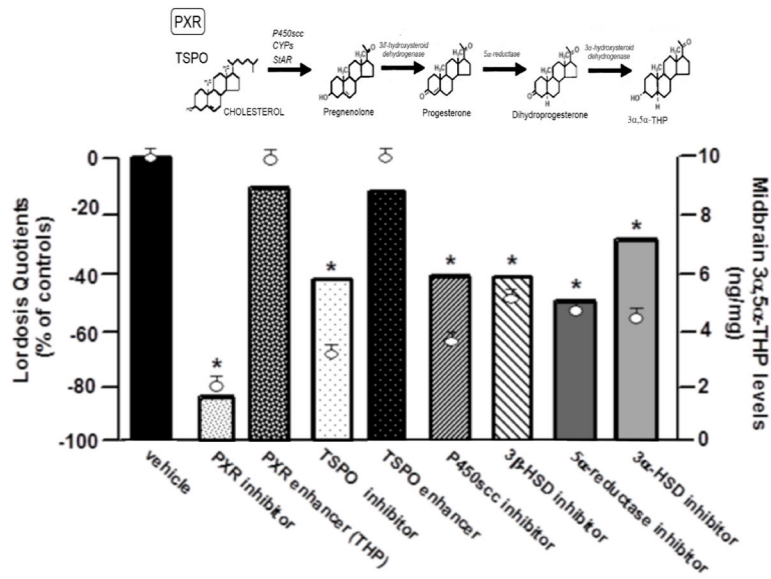


Figure 3. Intra-ventral tegmental area (VTA) infusions of nPR and mPR α AS-ODN do not influence lordosis, but lordosis is reduced by infusions of mPR β AS-ODN to the VTA * indicates significant difference from control infusions