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Novel substrates for, and sources of, progestogens for reproduction

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Abstract

Steroid hormones, such as progesterone (P), are typically thought of as being primarily secreted by the gonads (albeit adrenals can also be a source) and having their actions through cognate intracellular progestin receptors (PRs). Through its actions in the midbrain Ventral Tegmental Area (VTA), P mediates appetitive (exploratory, anxiety, social approach) and consummatory (social, sexual) aspects of rodents' mating behavior (Frye et al., 2006a). However, P and its natural metabolites ("progestogens") are produced in the midbrain VTA independent of peripheral sources and midbrain VTA of adult rodents is devoid of intracellular PRs. One approach we have used to understand P's effects and mechanisms in the VTA for mating is manipulate P's actions in the VTA and to examine effects on lordosis (the posture female rodents assume for mating to occur). This review focuses on the effects and mechanisms of progestogens to influence reproduction and related processes. Actions of P and its 5 α -reduced metabolite and neurosteroid, 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP; allopregnanolone) in the midbrain VTA to facilitate mating is described. The findings that 3 α ,5 α -THP biosynthesis in the midbrain occurs with mating are discussed. Evidence for 3 α ,5 α -THP's actions in the midbrain VTA via non-traditional steroid targets is summarised. The broader relevance of these actions of 3 α ,5 α -THP, for aspects of reproduction, beyond lordosis, are summarised. Finally, the potential role of the pregnane xenobiotic receptor in mediating 3 α ,5 α -THP biosynthesis in the midbrain is introduced.

Keywords

neurosteroid; progesterone; lordosis; midbrain ventral tegmental area; steroid biosynthesis; steroid metabolism

Introduction

Beyond their well-known effects in the body for maintaining pregnancy, progesterone (P) and its natural products (termed "progestogens"), such as 5 α -pregnan-3 α -ol-20-one (3 α ,5 α -THP; allopregnanolone), have robust actions at the level of the central nervous system (CNS). In addition to what can be considered these non-traditional effects of progestogens in the CNS, progestogens have sources and mechanisms that cannot be explained by the traditional dogma of what neuroendocrine signaling entails. Of interest is further

understanding the non-traditional effects of steroids in general, but a focus in this review will be novel actions of progestogens, which share characteristics with other steroids. Specifically, research from our laboratory is aimed at elucidating the effects, sources, and mechanisms by which progestogens mediate motivated behaviours, such as reproduction and other reproductively-relevant behaviours. A particular emphasis is on actions of progestogens in the midbrain ventral tegmental area (VTA), as described further in this review, to influence such motivated behaviours. To this end, the approach we first used to understand progestogens' effects and mechanisms in the VTA for mating was to manipulate progestogens' actions in this region and to examine effects on lordosis, the stereotypical mating posture that female rodents assume under appropriate hormonal and environmental contexts, as a bioassay. These studies have been followed up by investigating whether there are similar effects of progestogens in reproductively-relevant behaviours, such as aggression and affect, which will be discussed in this review. What follows is a review of some of the main results and recent findings regarding the sources and mechanisms of progestogens using this model to understand the role of progestogens for mating behaviour within a broader context of understanding non-traditional neuroendocrine signaling for other motivated behaviours.

Progestogens as neurosteroids and their functional effects

Neurosteroids are steroids that are synthesised in the CNS, rather than peripheral glands (gonads, adrenals, and/or placenta; 1, 2). Levels of neurosteroids are typically greater in the CNS than in circulation (1, 2). Enzymes involved in peripheral gland steroidogenesis have been identified in the CNS (3, 4). As well, high CNS levels of neurosteroids persist after surgical removal of peripheral glands (gonadectomy-GDX and/or adrenalectomy-ADX; 1, 2, 5–7). Thus, some neurosteroids in the CNS of rodents are produced by local biosynthesis.

De novo production of progestogens in brain

Progestogens can be formed centrally from biosynthesis and/or metabolism. The brain, like the adrenals, gonads, and placenta, is an endocrine organ and can produce neurosteroids independent of peripheral glands to form neuroactive products (neuroactive steroids; 1, 2, 5–8). The BZRP gene mediates the expression of the translocator protein (18 kDa; TSPO, previously known as the peripheral-type benzodiazepine receptor) that is widely expressed throughout the body, particularly in steroidogenic tissues (9, 10). TSPOs import cholesterol into the mitochondria, whereupon it is oxidised to pregnenolone by two proteins that initiate steroidogenesis – the steroidogenic acute regulatory (StAR) protein and cytochrome P450-dependent C27 side chain cleavage enzymes (P450_{scc}), rate-limiting steps in steroid biosynthesis, or production of steroids from the precursor, cholesterol (11–13). The latter involves a nuclear pregnane xenobiotic receptor (PXR) that regulates gene transcription for cytochrome P450 enzymes, which may be necessary for biosynthesis of 3 α ,5 α -THP (14–16). Pregnenolone is converted by 3 β -hydroxysteroid dehydrogenase (3 β -HSD) to P. P, from central or peripheral sources, is metabolised irreversibly by 5 α -reductase (5 α -R) to dihydroprogesterone (DHP), followed by actions of 3 α -hydroxysteroid dehydrogenase enzymes (3 α -HSD), to form 3 α ,5 α -THP (2). P is also a precursor of androstenedione and deoxycorticosterone (2). Levels of brain enzyme expression are highest in midbrain, limbic regions (cortex, hippocampus, basal ganglia, hypothalamus, thalamus), cerebellum, tectum, pons, medulla, spinal cord, and pituitary (3, 4, 17–19).

Progestogens' organizational and activational effects for reproduction

Hormones, secreted by the gonads, may have both organizational and activational effects. Organizational effects of hormones occur during critical periods of development (typically pre- or peri-natally) and can result in permanent changes in CNS structure (i.e. sexually

dimorphic areas of the hypothalamus) and/or function (hypothalamic–pituitary–gonadal (HPG) and/or adrenal (HPA) axes; 21). These changes can alter physiological and/or behavioural processes among females and males. P and its natural metabolites (DHP and 3 α , 5 α -THP) exert profound activational and/or organizational effects. Among adult female rodents, a salient activational effect of progestogens is evidenced when levels are increased around ovulation, and in response to mating-relevant stimuli (22–24). This enhancement of progestogens facilitates mating behaviour and promotes expression of the stereotypical lordosis posture in response to a sexual contact among adult female rodents (8, 22–24). Research from our laboratory is focused on the sources and mechanisms of progestogens in the midbrain VTA. To this end, reproductive responding (lordosis) is utilized as a bioassay to further understand progestogens' role in the midbrain VTA.

Biosynthesis of progestogens in the midbrain to facilitate mating behaviour

A question is the source of progestogens for reproductive behavior. Whether increased levels of 3 α ,5 α -THP in midbrain are due to central biosynthesis and/or dependent upon ovarian sources was of interest. The midbrain VTA is one brain target of interest given its key role in sexual behavior and other processes mediated by the dopaminergic system and because there is high expression of steroid biosynthesis and metabolism enzymes in this region. To investigate the role of central biosynthesis versus metabolism of ovarian P in the midbrain VTA for mating, pro-oestrous rats were infused with inhibitors of 3 α ,5 α -THP formation to the midbrain VTA, such as PK11195 (an inhibitor of TSPO), indomethacin (an inhibitor of 3 α -HSD), and/or vehicle. PK11195 inhibits 3 α ,5 α -THP formation from cholesterol, while indomethacin blocks DHP metabolism to 3 α ,5 α -THP. Infusions of any combination of inhibitor significantly attenuated midbrain 3 α ,5 α -THP levels of pro-oestrous rats concomitant with reductions in sexual behaviour (20). In order to assess whether central 3 α ,5 α -THP is necessary and sufficient for these effects, pro-oestrous rats were infused with 3 α ,5 α -THP subsequent to inhibitor infusions and results indicated a reinstatement of sexual behaviour that was commensurate to that of vehicle-infused controls (20). In a follow-up study, behavioural effects of infusions of a neurosteroid enhancer (FGIN 1,27, a TSPO agonist) following inhibitor infusion was assessed and revealed that enhancement of natural central biosynthesis could overcome effects of 3 α ,5 α -THP inhibitors (8). Thus, these data suggest that central biosynthesis of 3 α ,5 α -THP in the VTA is necessary and sufficient to enhance expression of sexual behaviours in pro-oestrous rats.

Progestogens' traditional mechanisms of action involving progestin receptors (PRs)

Actions of steroids through nuclear PRs

Nuclear steroid receptors are typically found in the nucleus or cytoplasm of cells. These intracellular receptors are activated when hydrophobic steroids freely diffuse through cell membranes and bind to them. The steroid-receptor complex then translocates to the nucleus, where it binds to hormone response elements on genes, which changes the rates of transcription and translation (25). This traditional “genomic” action of steroids involving transcription of DNA and synthesis of protein is a relatively slow process, taking minimally 10 min, but more often hours to days, to elicit a biological response (26, 27).

PRs are intracellular, cognate, steroid receptors for P that were first characterised nearly 40 years ago (28). As with other steroid receptors in the nuclear receptor superfamily, PRs bind ligands and act as transcription factors to evoke expression of mRNAs leading to changes in protein synthesis, and, thereby, biological actions (29, 30). In their unliganded state, PRs complex with chaperone molecules, such as several heat shock proteins, which is necessary for binding in the presence of P (31, 32). When bound by ligand, there are conformation

changes in the PRs, dimerization, and interactions with response elements of PRs (PREs) in the promoter regions of target genes (33–36). However, these PREs are not always required to instate gene-mediated changes in protein synthesis (37–39).

Progesterone's actions via PRs in the hypothalamus to facilitate initiation of lordosis

Although P can have many behavioural effects, one focus has been on its role in reproductive behaviours, such as lordosis. P can have actions through PRs in the ventromedial hypothalamus (VMH) to facilitate lordosis, and expression of PRs is increased coincident with initiation of sexual receptivity (29). Activation or knockdown of PRs in the hypothalamus using PR antagonists, anti-sense oligodeoxynucleotides (ODNs) to PR mRNA, or PR gene disruption in rodents, demonstrate initiation and attenuation of lordosis, respectively (40–47). These, and other findings, suggest that PRs are critical for initiation of P-facilitated reproductive behaviours, such as lordosis.

Progesterone non-classical actions involving PRs

There are also “non-genomic” mechanisms involving PRs and activation of second messenger signalling molecules (48, 49). Indeed, PRs are expressed in many regions of the CNS (e.g. amygdala, bed nucleus of the stria terminalis, hypothalamus, hippocampus, cortex, preoptic area, the ventromedial and dorsomedial nucleus of the hypothalamus, arcuate nucleus, cerebellum; 50–59), in most cell types. There is some indication that both forms of PR expression (PR-A and PR-B) can vary by brain region or cell type, such as reproductive tissues, as a function of hormonal milieu (60–63). Sex differences and hormonal mediation of PR-A and PR-B expression has been observed in the olfactory bulbs, hippocampus, and cerebellum of rats (54,55). As well, non-genomic actions of progesterone in the hypothalamus may involve factors, such as calmodulin-dependent protein kinase II (64). Thus, it is possible that progesterone can have actions independent of classic mechanisms via PRs.

Actions of progesterone independent of nuclear PRs

Steroid hormones play fundamental roles in the development and/or function of the CNS; however, the effects and mechanisms of action associated with steroid hormones needs to be better understood. Steroids are typically considered to have actions through nuclear steroid receptors. In addition to the relatively slow, genomic actions that have traditionally characterised steroid-mediated effects, steroids can also exert rapid and “non-genomic” actions. Indeed, steroids can act on target tissues faster (from milliseconds to 10 minutes) than the time required for transcriptional activity of nuclear steroid receptors (26, 27). These rapid, “non-genomic” actions of steroids have been investigated over the past few decades and have been demonstrated for all the major classes of steroids (65–68). The effects, substrates, and mechanism of such “non-genomic” steroid actions are of great interest.

Progesterone seems to have actions independent of nuclear PRs in a number of systems. In gametes, there are rapid, non-genomic actions of progesterone, including rapid increases in sperm motility and in the induction of the sperm acrosomal reaction (69–74). Progesterone also has rapid effects on oocyte maturation in fish and amphibian species (75–77). In brain, there are rapid effects of progesterone on gonadotropin-releasing hormones (78–80). In addition, there is a rapid activation of breast cancer cell signaling (81) and signal transduction pathways in human T lymphocytes and Jurkat cells that lack nuclear PRs (82). Thus, there are many instances of progesterone exerting rapid effects independent of nuclear steroid receptors.

Progestogen's actions via PRs in the midbrain VTA are not required for lordosis

Observations of the relationship between progestogens in the midbrain VTA and lordosis quotient have been conserved across multiple rodent species, including rats, hamsters, and mice (Figure 1). In rodents, the facilitation of lordosis occurs, in part, by oestrogen (E) and P through actions via cognate PRs in the ventral portion of the VMH (83). The duration and intensity of lordosis is mediated by actions of P in the midbrain VTA (17, 18). However, there are very few intracellular PRs localised to the VTA of rodents in adulthood and these are not inducible by E (84–87). Infusion of PR anti-sense ODNs to the VTA does not alter lordosis, while manipulations directed to the VMH reduce lordosis (17, 18, 40, 88). However, intravenous or intra-VTA infusions of P to hamsters or rats, rapidly enhances firing of VTA neurons within 60 s (89–90) and facilitates lordosis within 2 minutes (91, 92). This occurs even when P's actions are relegated to cell membranes (93, 94). Thus, P's actions in the VTA to facilitate lordosis do not require classic actions via intracellular PRs.

Actions of progestogens in the midbrain VTA via non-traditional steroid targets to facilitate mating behaviour

Manipulating P's actions in the VTA of naturally sexually-receptive, or ovariectomised (OVX), E-primed rodents, and observing changes in sexual responding, has elucidated P's functionally-relevant mechanisms for mating. In the VTA, P's metabolite, $3\alpha,5\alpha$ -THP, has actions to facilitate mating (17, 18, 95, 96). $3\alpha,5\alpha$ -THP is devoid of affinity for cognate, intracellular PRs, but can have agonist-like actions at γ -aminobutyric acid (GABA) receptors, dopamine type 1-like receptors (D_1), and antagonist-like actions at N-methyl-D-aspartate type glutamate receptors (NMDARs; 8, 40, 97–100) in the VTA to facilitate mating, as described in the next section.

It is beyond the scope of this review to exhaustively cover the plethora of non-steroidal targets that progestogens have. For example, $3\alpha,5\alpha$ -THP can have actions through other non-steroidal targets, which can include ligand-gated, ion channels and/or G protein-coupled receptors, such as glycine, glutamate (e.g. kainite, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid- AMPA), norepinephrine, dopamine, serotonin, acetylcholine, oxytocin and nicotinic/muscarinic receptors, as well as sigma type 1 and 2 receptors (101, 102). Indeed, we have investigated the effects of an oxytocin antagonist to the VTA. In this study, hormone-primed, OVX hamsters were administered the oxytocin antagonist, $t,d(CH_2)_5$, $[TYR(ME)_2,Thr(4),Tyr-NH(9,2)]$, and the time spent in lordosis during a 10-minute test with a sexually-experienced male was determined. The oxytocin antagonist attenuated lordosis (Figure 2), an effect similar to what has been observed among rats (103). Moreover, $3\alpha,5\alpha$ -THP can alter neuronal function of membrane E receptors (104) and G protein-coupled membrane PRs (105). Although neurosteroids have many targets, our focus has been on actions through $GABA_A$ receptors, NMDARs, and D_1 receptors, as described as follows.

Progestogens have actions via GABA to mediate lordosis

Progestogens have actions via the GABAergic system. Anatomical and biochemical studies indicate that the number, density, and affinity of $GABA_A$ receptors are modified by hormones. In support, number (106), density (107), and affinity (108) of $GABA_A$ receptors are enhanced during pro-oestrous compared to other phases of the oestrous cycle. The number of $GABA_A$ receptor binding sites also tended to be greater in the midbrain of hormone-primed, compared to OVX, hamsters (109). Levels of GABA are also increased in the midbrain of sexually receptive, compared to non-receptive, hamsters (17, 18). In a competitive [3H] muscimol (GABA mimetic ligand) binding assay, we observed that the concentration of GABA necessary to displace 50% of [3H] muscimol from its binding site, is

significantly less in the midbrain of hormone-primed and mated hamsters compared to OVX hamsters (109). Thus, progestogens can alter GABA levels and GABA receptors.

Through actions via GABA_A receptors in the VTA, progestogens mediate appetitive (exploratory, anxiety, social approach) and consummatory (social, sexual) aspects of rodents' mating behaviour (17, 18, 96). Infusions of muscimol, a GABA mimetic, to the VTA increases lordosis of hamsters or rats administered low dosages of systemic P (94, 110–112). Administration of the metabotropic GABA_B agonist, baclofen (which does not activate ionotropic GABA_A receptors), to the VTA enhances lordosis of OVX, E and P-primed rats (Figure 3). Pharmacological blockade of GABA_A receptors, via infusions of GABA_A antagonists, bicuculline or picrotoxin, to the VTA, attenuates P-facilitated lordosis of hamsters or rats compared to vehicle infused controls (40, 109, 111, 112). GABA is synthesised in neurons by the enzyme glutamic acid decarboxylase (GAD), from the precursor, glutamate, and is degraded by GABA-transaminase. Infusions of GAD antisense to the VTA of hormone-primed and pro-oestrous rats or hamsters disrupt lordosis, and reduce GAD immunoreactivity in the midbrain, compared to vehicle or sense infusions (88, 113). Infusion of valproate, a GABA-transaminase inhibitor, to the VTA of hamsters facilitates lordosis (111). Thus, enhancement and blockade of GABA_A receptors in the VTA of hormone-primed rodents mediates expression of lordosis, and may be influenced by progestogen actions at GABA_A receptors.

3 α ,5 α -THP is among the most effective ligands for GABA_A receptors. Structural requirements associated with progestogens that are the most effective positive modulators of GABA_A receptors include A-ring reduction at the 5 position (42), which is characteristic of 3 α ,5 α -THP. At nanomolar concentrations, 3 α ,5 α -THP alters the duration of GABA current (114). In nanomolar concentrations, 3 α ,5 α -THP directly activates GABA_A receptors and is a positive, allosteric modulator. 3 α ,5 α -THP increases chloride channel currents and lowers neuronal excitability with 20 and 200-fold higher efficacy than benzodiazepines or barbiturates, respectively (115–121). 3 α ,5 α -THP is approximately 60 times more potent than P at enhancing GABA_A receptor function (79). Compared to P, 3 α ,5 α -THP is more effective at rapidly inhibiting binding of the convulsant tert-butylbicyclophosphorothionate to the GABA-operated chloride channel (122), potentiating GABA's effects on chloride uptake (123), and increasing flunitrazepam binding (79). Infusion of progestogens to the VTA of rats and hamsters, resulting in facilitation of lordosis, corresponds to their ability to enhance GABA-stimulated chloride influx and SR 95531 receptor binding in the VTA (113). Thus, 3 α ,5 α -THP's lordosis-enhancing effects in the rodent VTA may occur in part via GABA_A receptors.

Progestogens have actions via NMDARs and D₁ receptors to mediate lordosis

Many of the GABAergic neurons that exist in the midbrain VTA also express NMDARs (124). Indeed, it has been proposed that post-synaptic glutamate receptors, such as NMDARs, AMPA/kainate, and metabotropic glutamate receptors, mediate reinforcing effects via their modulatory activity on DA cell bodies (125). Dopaminergic cell bodies in the VTA have glutamatergic and GABAergic inputs and co-localise NMDARs (126, 127). Given this neuronal circuitry in the midbrain VTA and findings that 3 α ,5 α -THP reduces glutamate's excitatory effects (128), this suggests that progestogens may also have actions via NMDARs and dopamine signaling.

NMDARs may be a substrate for progestogens' actions for lordosis. Autoradiography studies indicate OVX rats administered P and/or E have reduced NMDA binding in cortex (129, 130). Midbrain levels of glutamate are increased with P administration (17, 18). Antagonising NMDARs via intra-VTA infusions of MK-801 or LY235959 enhances progestogen-facilitated lordosis (99, 100). These data indicate actions of 3 α ,5 α -THP in the

VTA may occur, in part, through its antagonist-like actions at NMDARs. Presynaptic actions on the mesocorticolimbic dopamine pathway may be a key mechanism by which dopaminergic drugs and GABA_A receptor modulators (drugs or progestogens) achieve their effects on sex behaviour.

Dopaminergic neurons in the midbrain VTA are another substrate through which 3 α ,5 α -THP has actions to facilitate lordosis. Dopamine cell bodies are localised to the VTA, and activity of D₁ receptors can regulate their function (131). GABAergic terminals in the VTA, which have D₁, synapse on dopaminergic cell bodies that contain GABA_A receptors and NMDARs (126, 127). Increased GABA input onto GABA_A receptors that are located on GABAergic interneurons in the VTA (131) reduces the inhibitory actions of these cells on dopamine-containing cell bodies and increases somatodendritic dopamine release (133). This may account in part for enhancements in lordosis, produced by muscimol infusions to this region. Progestogens increase D₁ density in the striatum and dopamine levels in the midbrain (17, 18, 134). D₁ antagonists attenuate, and D₁ agonists enhance, progestin-facilitated lordosis when administered to the VTA (135, 136). Moreover, infusions to the VTA of the GABA_A receptor antagonist, bicuculline, attenuate the facilitatory effects of the D₁ agonist, SKF38393, and/or progestogens for lordosis (98). Another consideration to make is that several lines of evidence point to a role of sigma type 1 receptors in the activity of NMDA and D₁ receptors, suggesting a potential additional mechanism for progestogens to have actions through this pathway (137). Thus, progestogens may have effects in the midbrain VTA by modulating GABAergic and/or mesocorticolimbic dopamine function.

The role of signal transduction cascades for progestogens' actions

Downstream activation of signal transduction factors may underlie some of progestogens' actions at membrane targets, such as GABA_A receptors, NMDARs and/or D₁ (138, 139). D₁ receptors are well-known metabotropic receptors (140). Emerging evidence suggests that GABA_A receptors may also have actions involving signal transduction pathways. 3 α ,5 α -THP's effects to prolong the opening of GABA_A receptor chloride channels *in vitro* can be inhibited with antagonists of G-proteins, PKC, or cAMP-dependent protein kinase (141). Further, systemic administration of P increases levels of cAMP in the prefrontal cortex in a 3 α ,5 α -THP- and GABA_A receptor- dependent manner (17, 18, 95). Blocking activity of adenylyl cyclase, G-proteins, PKA, PLC, or PKC attenuates the enhancing effects of GABA_A receptors or D₁ agonists, or NMDAR antagonists, for 3 α ,5 α -THP-facilitated lordosis (135, 136, 141–150). Thus, progestogens' actions in the VTA involve activation of metabotropic pathways.

Dynamic actions of progestogens in the midbrain VTA to mediate expression of reproductive behaviours

In response to mating, 3 α ,5 α -THP levels in midbrain VTA, metabolised from ovarian P or as a result of neurosteroidogenesis, are rapidly increased compared to non-mated naturally-receptive or hormone-primed rodents (17, 18, 95, 151–153). The rapidity of the mating-induced increase in midbrain 3 α ,5 α -THP, and independence of secretion from the ovaries and/or adrenals, suggests that biosynthesis and subsequent metabolism of central, rather than peripheral, progestogens underlie mating-induced increases we have observed among rats, mice, and/or hamsters (17, 18). Mating-induced 3 α ,5 α -THP biosynthesis not only occurs in the midbrain, but also in the hippocampus, cortex, and striatum, areas of the brain involved in cognitive, affective, and motivated processes (151, 153). Thus, the effects and mechanisms underlying dynamic effects of 3 α ,5 α -THP to facilitate, and be increased by, mating are of great interest.

Progestogens may influence the propensity for expression of sexual receptivity in females, rather than instate receptivity behaviours. For example, progestogens do not seem to instate lordosis, but rather make it more likely that such a response will be evoked in the presence of mating-relevant stimuli (i.e. anogenital investigation and mounting by a male). Rodent models that are typically considered to elucidate whether a function may be steroid mediated are co-variation (oestrous cyclicity), surgical removal (OVX), and replacement (hormone-priming) studies. Thus, the following section describes evidence supporting the role of progestogens in the midbrain VTA to facilitate mating behavior, and reproductively-relevant behaviour, of rodents.

Endogenous increases in P and 3 α ,5 α -THP in the midbrain are associated with sexual receptivity

In the VTA, P has effects to facilitate lordosis subsequent to formation of 3 α ,5 α -THP (8, 17, 18). Indeed, there are co-variations between midbrain 3 α ,5 α -THP levels and lordosis. Mating and frequency in lordosis in female rodents coincides with ovulation, during the pro-oestrous phase of the oestrous cycle, when circulating and midbrain levels of progestogens, and other hormones in addition to progestogens, are elevated compared to the dioestrous phase (17, 18; Figure 4). This effect is attenuated among 12–14 month old, mid-aged rats, concomitant with progestogen decline (Figure 4). Higher midbrain 3 α ,5 α -THP levels and lordosis are observed among mid-aged rats that are not yet reproductively-senescent, compared to mid-age counterparts that are reproductively-senescent (154).

Removal and reinstatement of P and 3 α ,5 α -THP in midbrain VTA and sexual receptivity

The relationship between progestogens and lordosis has also been demonstrated through surgical removal and replacement studies. Lordosis and midbrain levels of 3 α ,5 α -THP are attenuated following OVX, but lordosis can be reinstated following systemic or midbrain VTA administration of P or 3 α ,5 α -THP to OVX, E-primed rats (17, 18; Figure 4). Administration of a 5 α -reductase inhibitor to the VTA results in a reduction in P's conversion to 3 α ,5 α -THP and lordosis is attenuated (similar to that observed with inhibitors of steroid biosynthesis or metabolism; 94, 97, 155–157; Figure 4). These effects may be specific to the VTA. Progestogen administration to sites nearby the VTA (such as the central grey, red nucleus, or substantia nigra) does not evoke facilitation of lordosis (151, 152). As well, application of biosynthesis or metabolism enzyme inhibitors to sites nearby the VTA does not produce decrements in P-facilitated lordosis (156). Thus, formation and/or actions of 3 α ,5 α -THP in the midbrain VTA may play an important role in the facilitation of lordosis.

Progestogens in the midbrain VTA mediate expression of reproductively-relevant behaviours

Given the role 3 α ,5 α -THP may play in reproductive behaviours, it is of interest to elucidate the role of 3 α ,5 α -THP for behavioural processes relevant for reproduction, such as social interactions, affect, and/or cognition. As a key brain region in the reward pathway, the VTA is involved in motivated behaviors beyond lordosis, such as aggressive, defensive, and affective behaviors. Indeed, 3 α ,5 α -THP has been implicated in the etiology, expression, and/or therapeutic treatment of anxiety, stress/mood, and cognitive (dys)regulations (8). By investigating behaviours commonly disrupted in neuropsychiatric disorders, such as sexual and social functions, and using an ethologically-relevant model of rodent reproductive behaviour, we hope to provide insight into the functional role of 3 α ,5 α -THP.

3 α ,5 α -THP & aggressive behaviour

Aggressive and defensive behaviours normally exhibited by female rodents toward male rodents must be dampened so that successful mating can occur, which suggests a role of

progesterones in the VTA, which are high with mating, for these motivated behaviors. The frequency of aggressive behaviors displayed by pro-oestrous rats are decreased compared to that of dioestrous rats (17, 18, 158), and coincident with increased $3\alpha,5\alpha$ -THP levels in the midbrain (Figure 5). Social isolation increases aggressive behavior of isolated rodents toward an intruder and drastically reduces $3\alpha,5\alpha$ -THP levels (159, 160). Fluoxetine can normalize social interactions and reverse decline in $3\alpha,5\alpha$ -THP (161). $3\alpha,5\alpha$ -THP, like other compounds that exert agonist-like effects at GABA_A receptors, has biphasic effects on aggressive behaviors. At moderate dosages, $3\alpha,5\alpha$ -THP increases aggression of mice beyond species-typical, adaptive levels (162). At high levels, $3\alpha,5\alpha$ -THP decreases aggressive behavior and produces sedation of mice (162). Moreover, blocking actions of 5α -reductase reverse effects of neurosteroids, such as $3\alpha,5\alpha$ -THP and 3α -androstenediol, for aggressive behaviors (163–165).

$3\alpha,5\alpha$ -THP & affective behaviour

As with aggressive and defensive behaviors, it is proposed that anxiety and fear behaviors normally exhibited by female rodents are dampened by progesterones so that successful mating can occur. In support, concomitant with enhancement of sexual receptivity, pro-oestrous females have decreased anxiety- and depression-like behavior compared to their dioestrous counterparts (166, 167), coincident with increased $3\alpha,5\alpha$ -THP levels in the midbrain (Figure 6). OVX or inhibition of P's metabolism to $3\alpha,5\alpha$ -THP via 5α -reductase attenuates these enhancements (166, 168; Figure 6). Enhancing $3\alpha,5\alpha$ -THP can produce anti-anxiety-like effects (169–171).

PXR as a source of $3\alpha,5\alpha$ -THP biosynthesis in the VTA

PXR may be a target in the midbrain for $3\alpha,5\alpha$ -THP biosynthesis, relevant for motivated behaviors. PXR is a type II nuclear receptor (subfamily 1, group I, member 2; NR1I2) of average size (431 amino acids) that remains in the nucleus regardless of whether or not it is bound, and it must be agonised in order to promote gene transcription (172, 173). PXR is involved in the metabolism of cholesterol to bile acids and steroids (172). When PXR heterodimerises with retinoid X receptor (RXR), it regulates gene transcription in a ligand-dependent fashion. This promotes the production of many proteins, including cytochrome P450 enzymes (CYP enzymes), which are responsible for metabolism of several drugs and steroids (174, 175). PXR is most often considered in the liver, where it exists in relatively high concentrations (176). Indeed, PXR's specific functions in most parts of the brain are unknown; however, PCR and Western Blotting has revealed PXR in rat brain capillaries (177), and various regions of the human brain (178), and the rabbit cortex, midbrain, and cerebellum (176). In our laboratory, pilot studies have confirmed the expression of the PXR gene, RNA, and protein in the midbrain of rats in proestrus, as per microarray, reverse transcriptase polymerase chain reaction, and Western blots. Thus, we are interested in elucidating the role of PXR in $3\alpha,5\alpha$ -THP biosynthesis in the midbrain VTA.

PXR ligands enhance consummatory sexual behaviour

We have begun to assess the functional significance of actions at PXR in the VTA for effects on mating. OVX Long-Evans rats were stereotaxically implanted with bilateral guide cannulae aimed at the VTA. Rats were E-primed (10 μ g) and, 44 hours later, infused (1 μ l) with either a positive PXR positive modulator, $3\beta,5\alpha$ -THP (200 ng), $3\alpha,5\beta$ -THP (200 ng), $3\alpha,5\alpha$ -THP (200 ng), RU-486 (135 ng), or β -cyclodextrin vehicle (n=6/grp). Ten minutes later, rats were assessed for sexual receptivity. Preliminary results indicate that lordosis was increased among rats infused with any of the PXR- positive modulators, and was significantly greater among $3\alpha,5\alpha$ -THP- or RU-486- infused rats compared to vehicle-infused controls (Figure 7).

Blocking PXR in the VTA attenuates sexual behaviour

Although the preliminary data above imply that activating PXR in the midbrain VTA may facilitate lordosis, the consequence of PXR knockdown should be investigated to further elucidate the potential role of PXR in the midbrain VTA. OVX, E-primed (10 μ g) rats were infused with either a PXR anti-sense ODN (250 ng; n=15), a scrambled mis-sense ODN (250 ng; n=8) control, or β -cyclodextrin as a vehicle control (n=8) bilaterally to the VTA. ODNs were infused 44h and 24h prior to testing for paced mating. Ten minutes before testing, rats were infused with FGIN1–27 (5 μ g), a neurosteroidogenesis enhancer that is an agonist of TSPO. As we have previously observed, infusions of FGIN 1–27 to the VTA significantly enhanced lordosis. Infusions of anti-sense ODNs to the VTA significantly reduced lordosis and attenuated FGIN 1–27's effects (Figure 8). Thus, PXR may be important for facilitation of neurosteroid-enhanced lordosis.

Summary

This review focused on emerging findings using lordosis as a behavioural bioassay for progestogens' actions in the midbrain VTA to challenge traditional dogma in neuroendocrinology and reveal novel substrates for, and sources of, progestogens' actions. Progestogens, as with other steroid hormones, can act in the CNS via membrane or rapid-signalling actions, which occur within seconds to minutes. Progestogens can produce rapid effects on neuronal excitability and synaptic function that involve direct or indirect modulation of ion-gated, or other neurotransmitter receptors and transporters, rather than classic, nuclear hormone receptors (Figure 9). Further, the sources of these progestogens are not only the gonads, adrenal and placenta, but also the brain, which acts as an endocrine organ that requires coordinated actions of steroidogenic enzymes in neurons and glia in different parts of the CNS to metabolise peripheral steroids to neuroactive products (neuroactive steroids) or to produce steroids *de novo* in the brain independent of peripheral gland secretion (neurosteroids). A novel target of biosynthesis may be PXR (Figure 9). Through its actions in the midbrain VTA, progestogens mediate appetitive (exploratory, anxiety, social approach) and consummatory (social, sexual) aspects of rodents' reproductive behavior. Moreover, there is broader relevance for understanding the actions and sources of progestogens, such as 3 α ,5 α -THP, for aspects of reproduction, beyond lordosis, such as for other motivated behaviors (aggression, affect, etc). Indeed, understanding these non-traditional actions of pregnane (neuro)steroids has relevance for other steroid hormones that have rapid actions through non-steroidal targets for their broad functional effects (e.g. estrogens, androgens).

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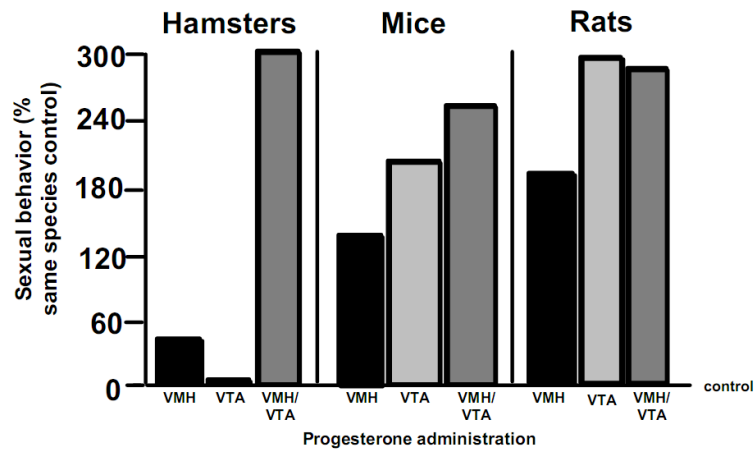


Figure 1.

There are species differences in dependence on progesterone (P) for enhancement of lordosis via direct administration to the ventromedial hypothalamus (VMH) and/or ventral tegmental area (VTA) of the midbrain, across rodents. Data shown are percentage of sexual behavior of rodents administered P compared to those administered oestrogen (E) subcutaneously. Rats have greatest response to P to the VTA and/or VMH, mice have a moderate response to P to VTA and/or VMH, and hamsters require P to both the VTA and VMH for sexual behavior.

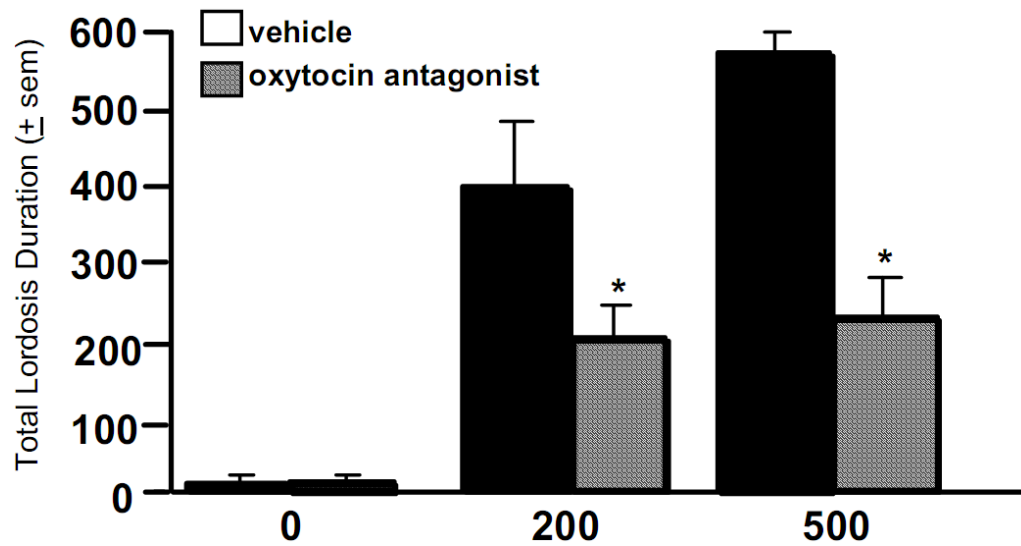


Figure 2. Subcutaneous injections of progesterone (P) dose-dependently (500 > 200 µg) enhances the duration of lordosis among ovariectomised hamsters. This effect is attenuated with subsequent administration of an oxytocin antagonist, t, d(CH(2))(5), [TYr(ME)(2), Thr(4), Tyr-NH(9,2)], via bilateral infusions to the ventral tegmental area (VTA), compared to saline vehicle infusions. * indicates significantly different from vehicle-administered controls, $p < 0.05$.

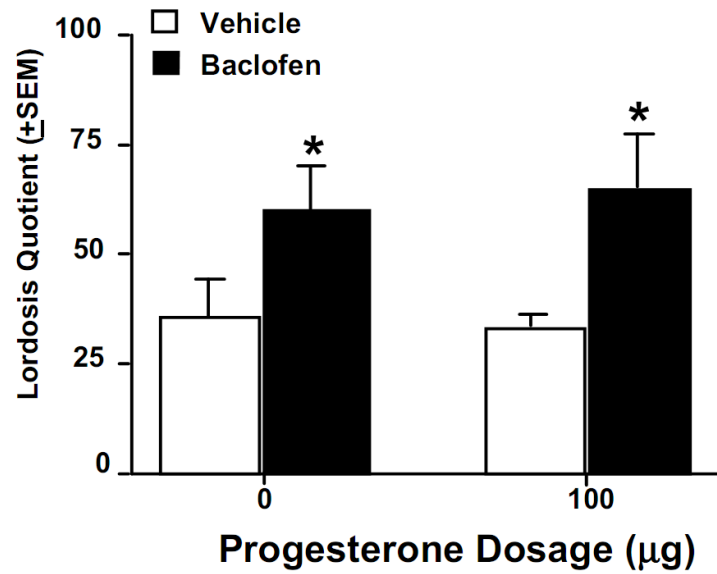


Figure 3. Systemic administration of the GABA_B agonist, baclofen, enhances lordosis among ovariectomised, oestrogen (E)- and progesterone (P)-primed, rats compared to vehicle administration. * indicates significantly different from vehicle-administered controls, $p < 0.05$.

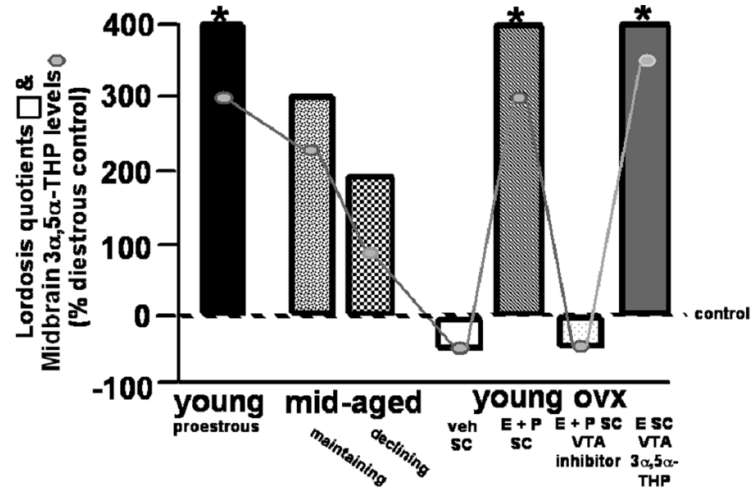


Figure 4.

Lordosis quotients (indicated in bars) are enhanced when midbrain concentrations of 3 α ,5 α -THP are increased either cyclically in young rats on pro-oestrus, in mid-age when reproductive status is maintained, when progesterone (P) is administered systemically after ovariectomy (OVX), or when 3 α ,5 α -THP is infused to the midbrain ventral tegmental area (VTA) compared to when midbrain levels of 3 α ,5 α -THP are low (as occurs during dioestrus, decline in reproductive status in mid-age, following OVX, or when P metabolism in the VTA is inhibited). Midbrain levels of 3 α ,5 α -THP of rats in these conditions are indicated by connected circles, as compared to dioestrous controls. * indicates significantly different from dioestrous controls, $p < 0.05$.

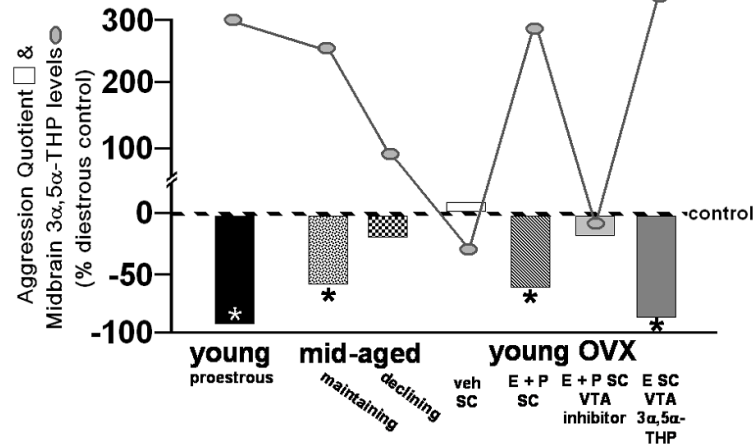


Figure 5.

Female aggression, in response to mounting by a male, is reduced when midbrain concentrations of $3\alpha,5\alpha$ -THP are enhanced either cyclically on pro-oestrous among young rats, in mid-age when reproductive status is maintained, when progesterone (P) is administered systemically after ovariectomy (OVX), or when $3\alpha,5\alpha$ -THP is infused to the midbrain ventral tegmental area (VTA) compared to when midbrain levels of $3\alpha,5\alpha$ -THP are low (as occurs during diestrus, decline in reproductive status in mid-age, following OVX, or when P metabolism in the VTA is inhibited). Midbrain levels of $3\alpha,5\alpha$ -THP of rats in these conditions are indicated by connected circles, as compared to dioestrous controls. * indicates significantly different from dioestrous controls, $p < 0.05$.

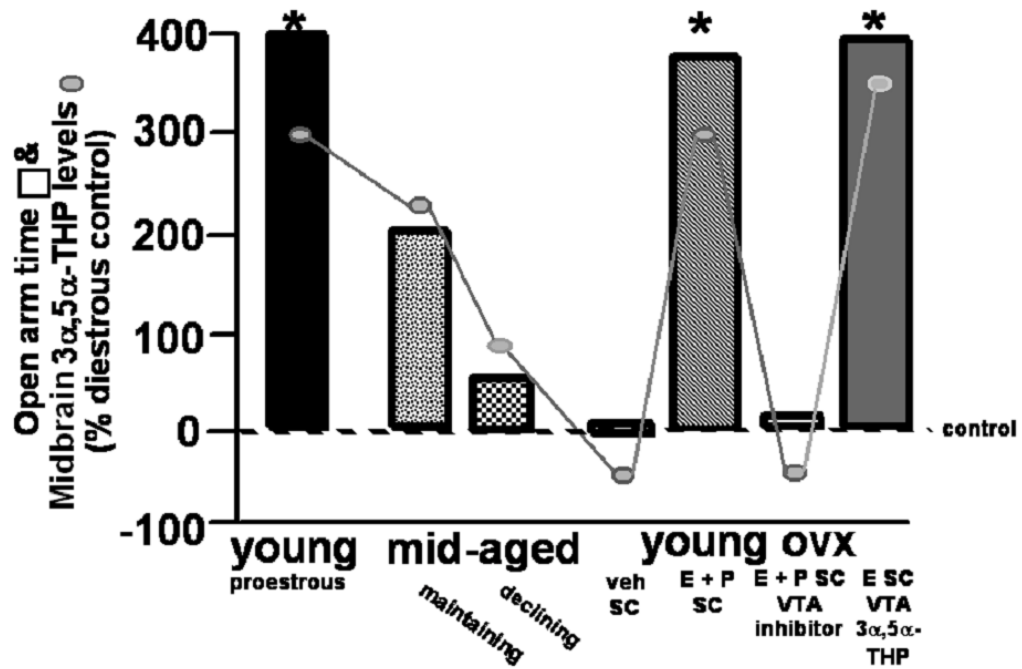


Figure 6.

The amount of time spent on the open arms of an elevated plus maze is increased when midbrain concentrations of 3α,5α-THP are enhanced either cyclically on pro-oestrous, in mid-age when reproductive status is maintained, when progesterone is administered systemically after ovariectomy (OVX), or when 3α,5α-THP is infused to the midbrain ventral tegmental area (VTA) compared to when midbrain levels of 3α,5α-THP are low (as occurs during diestrus, decline in reproductive status in mid-age, following OVX, or when P metabolism in the VTA is inhibited). Midbrain levels of 3α,5α-THP of rats in these conditions are indicated by connected circles, as compared to dioestrous controls. * indicates significantly different from dioestrous controls, $p < 0.05$.

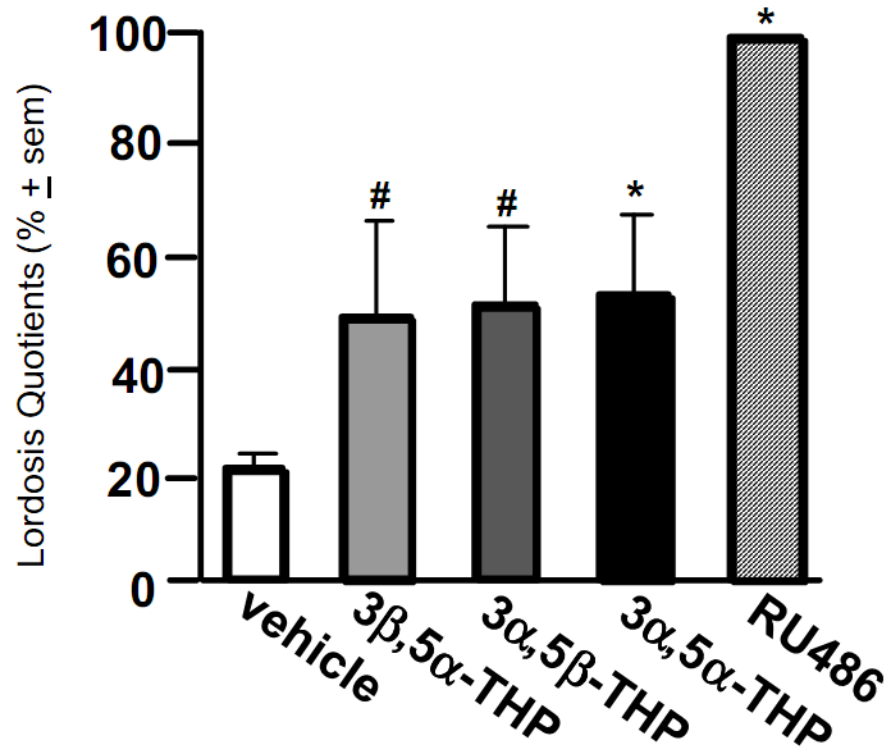


Figure 7. Intra-ventral tegmental area (VTA) infusions of positive pregnane xenobiotic receptor (PXR) modulators enhance lordosis. Infusions of 3 β ,5 α -THP (200 ng) and 3 α ,5 β -THP (200 ng) tended to increase lordosis quotients, and infusions of 3 α ,5 α -THP (200 ng) and RU486 (135 ng) significantly increased lordosis quotients among ovariectomised, oestrogen-primed rats. # indicates tendency to differ from vehicle-infused controls, $p < 0.10$. * indicates significant difference from vehicle-infused controls, $p < 0.05$.

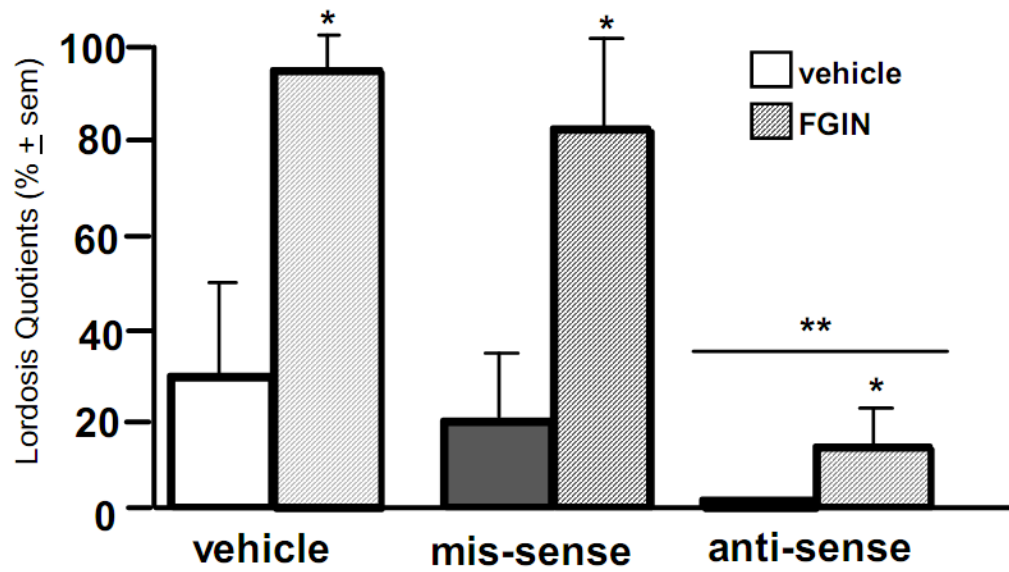


Figure 8.

Intra-ventral tegmental area (VTA) infusions of the neurosteroidogenesis enhancer, FGIN 1–27, significantly enhanced lordosis quotients among ovariectomised, hormone-primed, female rats ($n=3-10/\text{grp}$) and infusions of anti-sense oligonucleotides to the VTA blocked this effect. * indicates significant main effect for FGIN 1–27 to enhance lordosis quotients compared to vehicle, $p < 0.05$. ** indicates significant main effect for anti-sense infusions to reduce lordosis quotients compared to mis-sense or vehicle-infusions, $p < 0.05$.

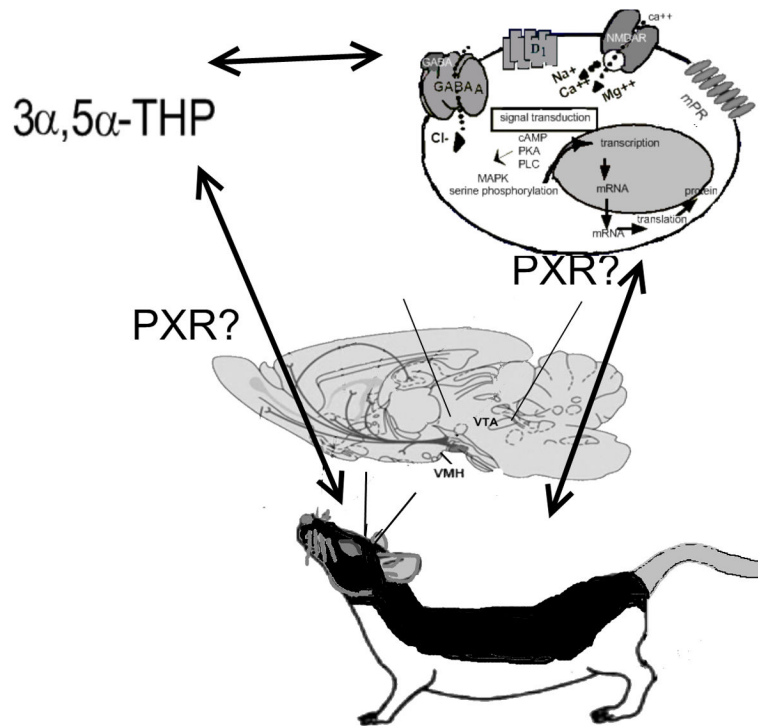


Figure 9.

Proposed relationship between pregnane xenobiotic receptor (PXR), $3\alpha,5\alpha$ -THP, and target substrates for actions to enhance lordosis. PXR may be involved in biosynthesis of $3\alpha,5\alpha$ -THP in the midbrain ventral tegmental area (VTA) with mating. Moreover, $3\alpha,5\alpha$ -THP is a ligand of PXR and, thus, PXR, like neurotransmitter targets (γ -aminobutyric acid- GABA receptors, N-methyl-D-aspartate receptors- NMDARs, and dopamine type 1-like receptors- D_1) and other membrane receptor targets may be important for actions of $3\alpha,5\alpha$ -THP in the VTA for lordosis.