Ligand-independent activation of progestin receptors: relevance for female sexual behaviour

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Traditionally, steroid receptors were believed to be activated only by ligand binding; however, recent studies indicate that steroid receptors can also be activated by mechanisms that do not require ligand, referred to as ligand-independent activation. Specifically, progestin receptors can be activated *in vitro* and *in vivo* after treatment with neurotransmitters, such as dopamine, in the absence of progesterone. Furthermore, mating-related stimuli can also lead to ligand-independent activation of progestin receptors in female rat brain. This finding indicates that environmental stimuli can influence steroid receptor-dependent processes in the absence of circulating hormone. This review focuses on studies indicating that progestin receptors can be activated in the absence of progesterone to influence neuronal response and sexual behaviour in rodents.

Ovarian steroid hormone receptors influence a variety of physiological and behavioural processes in many species, including humans. It had been assumed that intracellular steroid receptors were activated only by cognate ligands binding to a ligand-binding domain located on the receptor, referred to as ligand- or steroid-dependent activation. However, steroid receptors can be activated *in vitro* in the absence of steroid (Power *et al.*, 1991a; Aronica and Katzenellenbogen, 1993; Kazmi *et al.*, 1993), that is, by ligand- or steroid-independent activation. More importantly, ligand-independent activation of steroid receptors (that is, progestin receptors) within the brain can influence reproductive behaviour in rodents. This review focuses on recent findings which indicate that some somatosensory cues normally experienced by females, such as those associated with sexual contact with males, can activate progestin receptors to influence both neuronal response and oestrous behaviour in a ligand-independent manner.

Hormonal requirements for the induction of sexual behaviour

During the oestrous cycle in rats, sexual behaviour is dependent upon an increase in serum oestradiol followed by an increase in progesterone concentrations. Sexual behaviour is abolished by ovariectomy, and reinstated by the injection of oestradiol followed 1 or 2 days later by progesterone. Treatment with oestradiol alone can induce some aspects of sexual behaviour, such as receptivity (for example, lordosis); however, oestradiol followed by progesterone administration induces the full complement of proceptive behaviours that occur during normal oestrus,

such as ear wiggling, hopping and darting behaviour (Blaustein and Olster, 1989). This finding indicates that both oestradiol and progesterone are critical for the occurrence of normal oestrous behaviour. Oestradiol and progesterone are secreted from both the ovaries and the adrenal glands (Shaikh and Shaikh, 1975). Steroid hormone secretion from the adrenal glands appears to be important for the normal timing of sexual behaviour in rats, as stress resulting from sham ovariectomy advances, and stress resulting from adrenalectomy delays, the onset of oestrous behaviour. In addition, the small increase in solicitation behaviour induced by oestradiol treatment alone is blocked by adrenalectomy. Therefore, sexual behaviour is influenced by secretion of steroid hormones not only from the ovary, but also from the adrenal glands.

Behavioural and physiological changes associated with mating stimulation

When mating occurs, the female rat experiences a variety of somatosensory and olfactory stimuli from the male that further influences female sexual behaviour (Pfaff *et al.*, 1994). For example, ovariectomized rats primed with low doses of oestradiol exhibit reduced lordosis in the presence of a sexually active male; however, repeated exposure of ovariectomized oestradiol-primed female rats to males will induce increased lordosis (Hardy and Debold, 1973). The increase in lordosis owing to repeated mating of oestradiol-primed female rats occurs within an hour, and this behavioural phenomenon is not dependent upon the adrenal glands (Foreman and Moss, 1977). Therefore, mating-related stimuli increase sexual receptivity in the absence of circulating progesterone. It is not clear which somatosensory and olfactory cues are important for mediat-

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Fig. 1. Classical mechanism for activation of progesterin receptors by progesterone. Progesterone passes through the cell membrane and binds to progesterin receptors either in the cytoplasm or the nucleus. Ligand-bound receptors translocate into the nucleus, where they bind to another ligand–receptor complex. This dimer complex then binds to a response element on DNA, and interacts with nuclear receptor co-regulatory proteins to alter gene transcription. PRE: progesterin response element; P: phosphorylated site.

ing these increases in sexual receptivity. One potential somatosensory pathway may be via stimulation of the flank and perineum, which occurs when the male clasps the female during copulation. Stimulation of these regions appears to be critical for the elicitation of lordosis (Pfaff *et al.*, 1994).

Another pathway mediating mating-induced increases in sexual receptivity may be via vaginal–cervical stimulation (VCS). VCS can induce many different behavioural and endocrine changes in female rats, including increased lordosis, pseudopregnancy, and termination of sexual receptivity. VCS increases 2-deoxy-D-glucose uptake, an index of increased metabolism, within the medial preoptic area, bed nucleus of the stria terminalis and midbrain central gray (Allen *et al.*, 1981). Sensory information provided by VCS gains access to the central nervous system via pudendal, pelvic, hypogastric and vagus nerves.

Ligand-dependent activation of progesterin receptors

Steroid hormones influence sexual behaviour by binding to intracellular steroid receptors (Blaustein and Olster, 1989), although some effects may be the result of steroid hormone interaction with membrane receptors. After ligand binding, steroid receptors undergo a conformational change that

allows the ligand–receptor complex to bind to a hormone response element (HRE) on DNA. Although steroid receptors may bind to DNA as a monomer, receptor dimers are more stable and bind more efficiently to HREs. Once the steroid–receptor complex binds to DNA, it can interact with various combinations of co-regulatory proteins to influence genomic transcription (McKenna *et al.*, 1999) (Fig. 1). Co-regulatory proteins that increase steroid receptor action are called co-activators, and those that decrease steroid receptor action are called co-repressors. Interaction of steroid receptors with nuclear receptor co-activators appears to be important for steroid receptor action *in vitro* (McKenna *et al.*, 1999), as well as within the brain (Auger *et al.*, 2000a). Some cellular outcomes of steroid hormone action within the brain are changes in second messenger systems, inducible transcription factors, peptide receptors, and neurotransmitter synthesis and release.

Neuroanatomical distribution of oestrogen and progesterin receptors

In rat brain, gonadal steroid receptor-containing neurones are heterogeneously expressed in particular neuroanatomical sites (Pfaff and Keiner, 1973). There are at least two

Fig. 2. Ligand-independent activation of progesterin receptors. Neurotransmitters (for example, dopamine) or peptides stimulate membrane receptors, resulting in increased phosphorylation of proteins. These newly phosphorylated proteins (for example, steroid receptor co-activator 1 (SRC-1)) result in the recruitment of progesterin receptors to form an active transcriptional complex, which can regulate gene expression in the absence of progesterone. PRE: progesterin response element; P: phosphorylated site.

types of oestrogen receptor: oestrogen receptor α (ER α) and oestrogen receptor β (ER β). ER β has a high degree of sequence similarity with ER α (Kuiper *et al.*, 1996). Although small amounts of ER α mRNA can be found in many regions of female rat brain, the greatest amounts occur within the medial preoptic area, bed nucleus of the stria terminalis, ventromedial hypothalamus, arcuate nucleus, amygdala, lateral septum and some regions of the midbrain (Simerly *et al.*, 1990; Shughrue *et al.*, 1997). The distribution of ER β mRNA in the forebrain appears to have an overlapping distribution with ER α mRNA with some important exceptions: ER β mRNA appears not to be present within the ventromedial hypothalamus and in smaller amounts than ER α mRNA within the arcuate nucleus; whereas, ER β mRNA is present in high concentrations within the paraventricular nucleus of the hypothalamus and hippocampus compared with ER α mRNA (Shughrue *et al.*, 1997).

Although progesterin-binding sites are found throughout female rat brain, treatment with oestradiol increases progesterin receptor concentrations primarily within the preoptic area and mediobasal hypothalamus (Blaustein and Olster, 1989). Oestradiol-induced progesterin receptor-immunoreactivity in rats is expressed within the preoptic area, the ventromedial hypothalamus, the arcuate nucleus

and a small number of cells in the posterodorsal medial amygdala. Expression of progesterin receptors occurs in two forms, progesterin receptor A and progesterin receptor B (Conneely *et al.*, 1987). These two isoforms are expressed differentially within the brain during development (Kato *et al.*, 1993) and over the oestrous cycle (Guerra-Araiza *et al.*, 2000). As progesterin receptor A and B differ in the N-terminal sequence, which conveys gene activation (Tora *et al.*, 1988), these isoforms are functionally distinct (Mulac-Jericevic *et al.*, 2000). Therefore, differential activation of progesterin receptor isoforms can lead to different functional outcomes.

Ligand-independent activation of progesterin receptors *in vitro*

Although steroid hormones influence a variety of behavioural and physiological processes, the activation of nuclear steroid receptors is ultimately the most important factor in most of these outcomes. Therefore, attention should be focused on how steroid receptor activity is regulated within the brain. Oestrogen receptors (Aronica and Katzenellenbogen, 1993) and progesterin receptors (Power *et al.*, 1991a) can be activated *in vitro* in the absence

of their respective ligand. For example, treatment with 8-bromo-cyclic adenosine monophosphate (8-Br cAMP), an activator of protein kinase A (PKA), which phosphorylates serine or threonine residues, activates progesterin receptors *in vitro* in the absence of progesterone (Denner *et al.*, 1990; Kazmi *et al.*, 1993). Chicken ovalbumin upstream promoter transcription factor (COUP-TF) is activated *in vitro* by the neurotransmitter dopamine (Power *et al.*, 1991b). In addition, progesterin receptors can be activated *in vitro* by neurotransmitters, such as dopamine (Power *et al.*, 1991a) (Fig. 2).

Although some studies indicate that progesterin receptors are phosphorylated in different manners by ligand-dependent versus ligand-independent pathways (Bai *et al.*, 1997), phosphorylation of progesterin receptors via these two pathways may not differ functionally (Chauchereau *et al.*, 1994; Bai *et al.*, 1997). Furthermore, phosphorylation of progesterin receptors by ligand-independent pathways is not critical for progesterin receptor activation (Bai *et al.*, 1997). This finding indicates that progesterin receptors are not the target of phosphorylation by ligand-independent pathways. Indeed, the targets of phosphorylation by 8-Br cAMP may be nuclear receptor co-activators, such as steroid receptor co-activator 1 (SRC-1) (Rowan *et al.*, 2000). Treatment of COS-1 cells with 8-Br cAMP leads to increased phosphorylation of SRC-1 at threonine-1179 and serine-1185. Blocking the phosphorylation of threonine-1179 and serine-1185 inhibits the interaction of SRC-1 with another co-activator, cAMP response element binding protein (CREB)-binding protein (CBP). Preventing the interaction of SRC-1 with CBP impedes the formation of an active transcriptional complex (Rowan *et al.*, 2000), indicating that the phosphorylation of SRC-1 is a critical step for the formation of an active transcriptional complex associated with progesterin receptors. Since SRC-1 was first characterized, many co-activators have been reported, such as SRC-2, SRC-3 and androgen receptor co-activator 70 (McKenna *et al.*, 1999). Phosphorylation of target proteins other than progesterin receptors, such as nuclear receptor co-activators, allows for more combinations of phosphorylated proteins to be recruited into a transcriptional complex. One outcome of having various combinations of phosphorylated proteins within a transcriptional complex might be to provide distinct functional consequences. Thus, it is possible that ligand-activated progesterin receptors function in a more discrete manner than do ligand-independent activated progesterin receptors, as a result of differential phosphorylation of nuclear receptor co-regulatory proteins.

Further support for the idea that ligand versus ligand-independent pathways function to alter gene expression or behaviour differentially comes from examining the phosphorylation of the progesterin receptor isoforms by 8-Br cAMP. The progesterin receptor A isoform is more sensitive to 8-Br cAMP-mediated phosphorylation than is the progesterin receptor B isoform (Kazmi *et al.*, 1993). As progesterin receptor isoforms are involved in different functional outcomes, increasing ligand versus ligand-independent

pathways may alter one isoform preferentially, leading to a unique functional outcome. Alternatively, ligand-independent pathways may function to enhance ligand activation of progesterin receptors. This possibility is supported by data indicating that combined treatment of steroid with a phosphorylation activator leads to a greater *trans*-activation response of progesterin receptors than that observed with treatment of either steroid or phosphorylation activator alone (Edwards *et al.*, 1993; Kazmi *et al.*, 1993).

Ligand-independent activation of progesterin receptors *in vivo*

Converging evidence indicates that ligand-independent activation of progesterin receptors is important for female sexual behaviour. Dopamine increases lordosis when infused into the medial preoptic area or ventromedial hypothalamus of oestrone-primed female rats (Foreman and Moss, 1979). As dopamine leads to activation of progesterin receptors in tissue culture (Power *et al.*, 1991a) and increases sexual behaviour in oestrogen-primed females rats, it was hypothesized that dopamine increases sexual behaviour via ligand-independent activation of progesterin receptors (Mani *et al.*, 1994a). Infusion of the dopamine (D₁) receptor agonist SKF 38393 into the third ventricle of oestradiol-primed rats increased lordosis, and prior treatment with progesterin antagonists blocked dopamine facilitation of lordosis. Dopamine facilitation of sexual behaviour was independent of adrenal steroid secretions, as these findings were replicated in dexamethasone-suppressed female rats. This finding indicates that pharmacological treatment with dopamine can lead to the activation of progesterin receptors in the absence of progesterone. Numerous other factors have been found that appear to activate progesterin receptors in a similar manner to influence lordosis in oestradiol-primed rats. For example, gonadotrophin releasing hormone, prostaglandin E₂, dibutyryl cAMP, nitric oxide, 8-bromo-cGMP and cocaine all increase lordosis in oestradiol-primed female rats, and these increases are blocked by the progesterin antagonist RU486 (Mani *et al.*, 1994b; Beyer *et al.*, 1997; Chu *et al.*, 1999). It is conceivable that additional neurotransmitters or peptides will be found that activate progesterin receptors to influence sexual behaviour, as factors that increase phosphorylation of proteins may result in increased lordosis in oestradiol-primed female rats. Further cross-talk between dopamine, progesterin receptors and the cannabinoid Δ^9 -tetrahydrocannabinol (THC) has been described in the regulation of female sexual behaviour (Mani *et al.*, 2001). As with dopamine, infusion of THC into the third ventricle of oestradiol-primed rats increased lordosis, and prior treatment with progesterin antagonists blocked THC facilitation of lordosis (Mani *et al.*, 2001). Both progesterone- and THC-facilitated lordosis can be blocked by infusion of antisense oligodeoxynucleotides that reduce the expression of either progesterin receptors or dopamine D₁ receptors (Mani *et al.*, 2001), indicating that pathways leading to

ligand-independent activation of progesterin receptors are important in ligand activation of progesterin receptors. That is, the phosphorylation of target proteins that are normally activated by ligand-independent mechanisms are likely to be involved in interacting with ligand-activated progesterin receptors.

Influence on behaviour of mating-related stimuli that activate progesterin receptors

A major question concerning ligand-independent activation of progesterin receptors is: when is this mechanism activated under normal circumstances? As described above, sexual receptivity is increased in oestradiol-primed female rats during interaction with a sexually active male, and this increase occurs in the absence of serum progesterone. Dopamine is released during mating stimulation (Matuszewich *et al.*, 2000) and is implicated in the activation of progesterin receptors in the absence of progesterone (Mani *et al.*, 1994a). Therefore, sexual receptivity may be increased before a surge in progesterone in the presence of a sexually receptive male. For example, ovariectomized–adrenalectomized, oestradiol-primed female rats show increased sexual behaviour in a repeated-mating test with a sexually active male. Each mating test takes 15 min and is separated by a 15 min non-exposure period. Sexual receptivity in control-treated female rats is increased by the third and fourth mating test; however, this increase is inhibited completely in rats injected with the progesterin antagonist RU486 (Auger *et al.*, 1997). Therefore, progesterin receptors appear to be important in mediating mating-induced increases in sexual behaviour even in the absence of circulating progesterone. This finding is consistent with the idea that mating interaction can lead to ligand-independent activation of progesterin receptors to alter behaviour. The specific cue responsible has not yet been identified, but it appears that some somatosensory information provided by VCS may be involved in ligand-independent activation of progesterin receptors. Manual VCS plus flank–perineum stimulation reliably induces lordosis at the time of stimulation. There is no difference in lordosis quotients between rats pretreated with the progesterin antagonist RU486 and rats treated with oil vehicle, indicating that a neuronal component that is not dependent upon progesterin receptors is activated by VCS. However, RU486-treated female rats placed in an arena with a sexually active male 15 min after VCS show lower sexual receptivity than do the oil-treated controls (Auger *et al.*, 1997). Although RU486-treated females have the capacity to show a similar degree of lordosis to that displayed by oil-treated rats receiving VCS, RU486-treated females show less sexual behaviour in the presence of a male. Furthermore, females that are masked to prevent intromissions do not show increased sexual receptivity after repeated mating, indicating that VCS is required for mating-induced lordosis (Bennett *et al.*, 2001). Taken together, these data indicate that increased sexual receptivity in response to somatosensory information provided by VCS

during mating is the result of ligand-independent activation of progesterin receptors. As mating stimuli rapidly increase (Bennett *et al.*, 2001) and subsequently decrease (Reading and Blaustein, 1984) lordosis in oestradiol-primed female rats, it is possible that mating-induced activation of progesterin receptors is involved in the normal timing of sexual receptivity.

Neuroanatomical sites involved in mating-induced activation of progesterin receptors

One method for investigating the site of mating-induced activation of progesterin receptors in the rat brain is to examine neuronal responses to mating-related stimuli. A widely used technique to identify neurones that respond to particular stimuli is the immunocytochemical detection of immediate early gene proteins (also known as inducible transcription factors), such as Fos. VCS increases the expression of Fos protein in a variety of brain regions, including the medial preoptic area, ventromedial hypothalamus, dorsomedial hypothalamus, bed nucleus of the stria terminalis, amygdala and the anterior-periventricular thalamic nucleus (Pfaus *et al.*, 1993; Tetel *et al.*, 1993; Auger *et al.*, 1996). Furthermore, VCS increases Fos expression in progesterin receptor-containing neurones within the medial preoptic area, arcuate nucleus and the ventromedial hypothalamus (Auger *et al.*, 1996). More importantly, prior treatment with the progesterin antagonist RU486 reduces VCS-induced Fos expression only in areas such as the medial preoptic area and the ventromedial hypothalamus containing high concentrations of progesterin receptors (Auger *et al.*, 1997). VCS-induced Fos immunoreactivity was not reduced by RU486 in areas such as the amygdala or the anterior-periventricular thalamic nucleus containing little to no progesterin receptor immunoreactivity. The reduction of VCS-induced Fos expression by RU486 is independent of circulating progesterone, as it occurs in ovariectomized–adrenalectomized oestradiol-primed female rats (Auger *et al.*, 1997). This finding indicates that some cellular responses associated with VCS are mediated via progesterin receptors, as blocking progesterin receptor activation impedes VCS-induced Fos expression.

Further evidence that VCS influences processes involved in ligand-independent activation of progesterin receptors within steroid receptor-containing areas comes from examining a dopamine- and cyclic AMP-regulated phosphoprotein, DARPP-32 ($M_r = 32\ 000$). DARPP-32 is phosphorylated on threonine34 in response to activation of dopamine D_1 , but not D_2 , receptors (Walaas and Greengard, 1984). DARPP-32 is involved in mediating both progesterone- and dopamine-facilitated lordosis in oestradiol-primed female mice, indicating that DARPP-32 is important for ligand-independent activation of progesterin receptors (Mani *et al.*, 2000). Oestradiol-primed mice with a targeted disruption of DARPP-32 do not show increased sexual receptivity after treatment with either progesterone or dopamine. One mechanism by which the phosphorylation of DARPP-32

Fig. 3. Model for mating-induced activation of progesterin receptors. Mating stimuli (for example vaginal–cervical stimulation (VCS)) cause the release of dopamine (DA) in progesterin receptor-rich areas, such as the medial preoptic area and ventromedial hypothalamus. Dopamine, acting on dopamine D₁ receptors, increases the activity of cAMP and protein kinase A (PKA), leading to increased phosphorylation of proteins, such as steroid receptor co-activator 1 (SRC-1) and dopamine- and cyclic AMP-regulated phosphoprotein (DARPP-32). Phosphorylation of DARPP-32 leads to the inhibition of phosphatase-1, which dephosphorylates proteins, and thereby potentiates the phosphorylation of other proteins. Phosphorylation of SRC-1 leads to the recruitment of progesterin receptors and other co-activators to form a transcriptional complex, which can increase gene transcription and alter behaviour. P: phosphorylated site; PRE: progesterin response element.

may influence ligand-independent activation of progesterin receptors is by allowing increased phosphorylation of other cellular proteins by inhibiting protein phosphatase 1, which is involved in dephosphorylating proteins. These data support the contention that proteins involved in ligand-independent activation of progesterin receptors also mediate ligand activation of progesterin receptors.

An antibody that detects the phosphorylated form of DARPP-32 was used to examine female rat brains 1 h after receiving VCS. The number of cells expressing phospho-DARPP-32 immunoreactivity was increased within the medial preoptic area and ventromedial hypothalamus (Meredith *et al.*, 1998), indicating that some of the somatosensory stimuli associated with VCS may influence cells within the ventromedial hypothalamus and medial preoptic area via a dopaminergic mechanism. Consistent with the location of DARPP-32, dopamine D₁ receptor binding (Boyson *et al.*, 1986), D₁ receptor immunoreactivity (Levey *et al.*, 1993), and D₁ mRNA (Zhou *et al.*, 1999) have been localized within the amygdala, preoptic area and ventromedial hypothalamus. Therefore, VCS may

activate progesterin receptors to influence neuronal responses and behaviour via a dopaminergic pathway (Fig. 3).

Ligand-independent activation of progesterin receptors: regulation of gonadotrophin secretion

Ligand-independent activation of progesterin receptors may also be involved in the release of FSH (Knox *et al.*, 1996) and LH (Levine, 1997) in female rats. Oestradiol increases the release of FSH and LH in ovariectomized female rats, and treatment with progesterin antagonists blocks these increases (Knox *et al.*, 1996; Levine, 1997). It is not clear how oestradiol mediates these increases, but one pathway may involve nitric oxide. Oestradiol increases nitric oxide synthase, and nitric oxide stimulates cGMP, which has been shown to activate progesterin receptors (Chu *et al.*, 1999). Therefore, it is possible that oestradiol influences the release of LH via stimulation of nitric oxide–cGMP pathways. Alternatively, oestradiol may act via a dopaminergic mechanism. For example, oestradiol treatment increases the expression of D₁ dopamine receptors in neonatal rat

Fig. 4. Influence of ligand versus ligand-independent activation of progestin receptors on H928 binding. In the absence of progesterone, the hinge region of the progestin receptor is recognized by the progestin receptor antibody, H928. In the presence of progesterone, the hinge region of the progestin receptor is not recognized by H928, probably as a result of a conformational change in the progestin receptor that obscures the hinge region after progesterone binding. When progestin receptors are activated via a ligand-independent mechanism, the progestin receptor either remains unaltered or assumes a slightly different conformation that allows the hinge region to be recognized by the H928 antibody. Alternatively, ligand-independently activated receptors may be recruited to the transcriptional complex in a manner that still allows H928 to gain access to the hinge region. The differential conformational state of progestin receptors may allow specific interactions with co-regulatory proteins, which may assemble to provide a unique transcriptional function. A/B, C, D and E: domains of the progestin receptor; AF-1: activation function 1 region; AF-2: activation function 2 region; COOH: C-terminal domain; DBD: DNA-binding domain; Hinge: hinge region; H928: H928 progestin receptor antibody; LBD: ligand-binding domain; NH₂: N-terminal domain; Ser: phosphorylated serine residue.

hypothalamic cell cultures (Lee *et al.*, 1999), and these receptors appear to be constitutively active and to generate cAMP, which may lead to the activation of progestin receptors. Although oestradiol can activate progestin receptors ligand-independently to alter gonadotrophin secretion, oestradiol does not activate progestin receptors to alter behaviour. That is, oestradiol administered in high doses can increase lordosis but this increase is not blocked by the progestin antagonist RU486 (Blaustein *et al.*, 1987).

Do ligand and ligand-independent activated progestin receptors differ?

As discussed above, the significance of ligand-independent activation of progestin receptors may be to induce a different functional outcome from that of ligand-activated progestin receptors. Support for this contention comes from studies using antibodies directed at particular sequences of the progestin receptor. One progestin receptor antibody, H928, is directed against the hinge region (corresponding to amino acids 523–536), a sequence common to both the A and B forms of the progestin receptor (Natraj and Richards, 1993; Park-Sarge *et al.*, 1995). The affinity of H928 for progestin receptors is decreased by the presence of

progesterone, indicating that ligand-bound receptors are not recognized by H928. That is, H928 immunoreactivity is decreased within the medial preoptic area and ventromedial hypothalamus 1 h after progesterone treatment (Auger *et al.*, 2000b), probably as a result of a conformational change in the receptor after ligand activation, as ligand bound receptors are still recognized by another progestin receptor antibody (DAKO Corp., Carpinteria, CA) directed at a different region of the receptor (Auger *et al.*, 2000b).

If mating stimuli, such as VCS, activate progestin receptors in the absence of progesterone, then it would be interesting to determine whether VCS also decreases H928 immunoreactivity. Although VCS decreased H928 immunoreactivity in the medial preoptic area of ovariectomized oestradiol-primed rats, VCS did not decrease H928 immunoreactivity within the forebrain of ovariectomized–adrenalectomized, oestradiol-primed rats. This finding indicates that when progestin receptors are activated by progesterone, a conformational change occurs so that the hinge region is not recognized by the H928 antibody. In contrast, the hinge region of progestin receptors activated by mating stimuli in the absence of progesterone either remains in or assumes a form that can still be recognized by

the H928 antibody. Therefore, it is possible that progesterone receptors undergo a particular conformational change depending on whether they are activated in a ligand-dependent or ligand-independent manner (Fig. 4). Whether the differential conformational state of progesterone receptors is involved in the unique regulation of specific genes or behaviour remains to be tested. If activation of progesterone receptors by mating stimuli occurs in the absence of progesterone, then it is meaningful to determine whether the effects of this activation on behaviour are similar to those that occur when the receptor is activated by progesterone. Some outcomes that appear to be dependent upon progesterone in oestradiol-primed female rats are proceptive behaviours, such as ear wiggling, hopping and darting behaviour (Blaustein and Olster, 1989).

Is there a role for progesterone that is synthesized *de novo* in the brain?

Both the ovaries and adrenal glands synthesize progesterone but little attention is given to the role of progesterone that is synthesized locally within the brain. Rats that have been ovariectomized and adrenalectomized still have low but detectable concentrations of progesterone within the brain (Corpechot *et al.*, 1993). Neurotransmitters or mating stimuli that increase sexual behaviour may alter the synthesis or release of neurally derived progesterone, indicating that 'ligand-independent' activation of progesterone receptors might be mediated via locally derived progesterone. However, data collected in studies using the H928 antibody do not support this alternative hypothesis. If mating stimuli caused the release of neurally derived progesterone, then H928 immunoreactivity should be decreased after mating stimulation as a result of ligand binding to progesterone receptors. Although VCS decreased H928 immunoreactivity in ovariectomized rats, VCS did not decrease H928 immunoreactivity in ovariectomized-adrenalectomized rats. These data indicate that neurally derived progesterone is not involved in mating-induced activation of progesterone receptors, but further testing is needed to rule out this possibility.

Conclusion

Taken together, these data support the contention that cues normally experienced during mating can gain access to the brain, where they influence progesterone receptor-dependent processes in the absence of progesterone. Therefore, environmental signals, such as photoperiodic, chemosensory and somatosensory stimuli, may influence steroid receptor-dependent processes in a ligand-independent manner. Although the data presented in this review focus on the role of ligand-independent activation of progesterone receptors in modulating female sexual behaviour, it is likely that ligand-independent activation of steroid receptors affects other types of behaviour or physiological process, such as learning, aggression and developmental plasticity of neurones. Indeed, oestrogen, androgen, glucocorticoid and

some orphan nuclear receptors are activated in a ligand-independent manner. It is not clear why there is an additional pathway to activate steroid receptors, although there may be several explanations for the functional relevance of ligand-independent activation of progesterone receptors. (1) Ligand-independent activation of progesterone receptors acts synergistically with progesterone. This contention has arisen from numerous studies showing that increased phosphorylation of proteins enhances ligand activation of progesterone receptors. (2) Ligand-independent mechanisms are an important factor in ligand activation of progesterone receptors. (3) Ligand-independently activated progesterone receptors differ functionally from ligand activated receptors. (4) Ligand-independent mechanisms are involved in regulating the activity of different progesterone receptor isoforms, resulting in different functional outcomes.

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