

Role of progesterone and its intracellular receptors in the sexual behavior of female rodents

ANGÉLICA COYOY¹, IGNACIO CAMACHO-ARROYO² AND CHRISTIAN GUERRA-ARAIZA^{3*}

¹Chemistry Faculty, Department of Biology, Universidad Nacional Autónoma de México, Mexico, D.F., Mexico;

²Unit for Investigation in Human Reproduction, Instituto Nacional de Perinatología-Facultad de Química, Universidad Nacional Autónoma de México, Mexico, D.F., Mexico; ³Medical Research Unit of Pharmacology, Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, Mexico, D.F., Mexico.

ABSTRACT

Studies of the processes that regulate female sexual behavior in rodents indicate that progesterone plays a key role in the display of such behavior. These studies include the action of progesterone and its metabolites, the characteristics of the expression and distribution of intracellular progesterone receptor isoforms in brain regions involved in sexual behavior, synthesis, turnover and activation of progesterone receptor, and intercommunication between genomic and non-genomic signals mediated by progesterone. In this review, some characteristics of these elements involved in female sexual behavior in rodents are addressed. (REV MEX ENDOCRINOL METAB NUTR. 2015;2:95-103)

Correspondencia autor: Christian Guerra-Araiza, christianguerra2001@gmail.com

Key words: Progesterone. Progesterone receptor. Female sexual behavior. Central nervous system.

RESUMEN

Los estudios de los procesos que regulan la conducta sexual femenina en roedores señalan a la progesterona como un elemento fundamental en el despliegue de dicha conducta. Estos estudios incluyen la acción de la progesterona y sus metabolitos, las características de la expresión y distribución de las isoformas del receptor a progesterona (RP) en regiones cerebrales que participan en la modulación de la conducta sexual, los procesos de síntesis, recambio y activación del RP, así como la intercomunicación de señales genómicas y no genómicas mediadas por la progesterona. En esta revisión se abordan algunas características de estos elementos que participan en la regulación del despliegue de la conducta sexual femenina en roedores.

Palabras clave: Progesterona. Receptor de progesterona. Conducta sexual femenina.

Correspondence to:

*Christian Guerra-Araiza

Av. Cuauhtémoc, 330

Col. Doctores, Del. Cuauhtémoc, C.P. 06720, México, D.F., México

E-mail: christianguerre2001@gmail.com

Received for publication: 03-02-2015

Accepted for publication: 10-03-2015

INTRODUCTION

Progesterone (P4) and its receptors participate in the regulation of diverse physiological processes in mammals¹⁻³. One such process is the display of female sexual behavior in rodents, essential for reproduction. In this review, we present the role of P4 and its receptors in the control of female sexual behavior.

PROGESTERONE

Progesterone is a steroid hormone involved in the regulation of several functions in vertebrates. It is mainly synthesized in the ovary, adrenal gland, placenta, and the central nervous system^{4,5}. Progesterone is involved in processes such as regulation of ovulation, maintenance of pregnancy, sexual behavior, inflammation, neuronal excitability, neuroprotection, neurogenesis, myelination, memory and learning, sleep, and development of brain tumors^{5,6}.

Progesterone, once in the bloodstream, can flow free or bound to plasma proteins such as albumin or globulins. It binds with high specificity and affinity to globulins⁷. The effects of P4 occur in the short, medium, and long term since it has several mechanisms of action⁸⁻¹⁰. It exerts its effects through genomic (classical) and non-genomic mechanisms (non-classical). The genomic mechanism involves the binding of P4 with its intracellular receptors (PR) and thus regulates the transcription of specific genes. Non-genomic mechanisms involve the action of P4 on the plasma membrane, second messenger systems, interaction with specific membrane receptors, ion channels, activation of phosphorylation cascades, and interaction with neurotransmitter receptors such as subtype A γ -aminobutyric acid (GABA_A)^{4,5,9,10}.

CHANGES IN CONCENTRATIONS OF PROGESTERONE DURING THE ESTROUS CYCLE

The rat estrous cycle is divided into four stages¹¹ characterized according to the concentration of

estradiol (E2), P4, follicle-stimulating hormone, and luteinizing hormone¹², and the abundance of different cell types found in the vaginal surface. The duration of the estrous cycle is considered between 4.4 and 4.8 days. During the estrus and metestrus, E2 blood concentration is basal, while at night of diestrus an increase^{3,10,13,14} is presented to its maximum concentration at noon of proestrus (50 ng/ml). This increase activates the surge of gonadotropin-releasing hormone (GnRH) from the hypothalamus, which in turn triggers the secretion of luteinizing hormone by the hypophysis. During the afternoon of proestrus, E2 concentration rapidly decreases to baseline levels in the early hours of the morning of estrus^{3,10,13,14}.

The significant increase in the concentration of luteinizing hormone in proestrus is followed by an increase in P4 concentration (50 ng/ml). A few hours after this increase, ovulation and lordosis (characteristic sexual behavior) are presented and subsequently copulation can occur in the morning of estrus. During the estrous cycle, P4 concentration increases again around noon of metestrus (25 ng/ml), extends until the morning of diestrus, and returns to baseline concentrations in the afternoon of that same day¹³.

PROGESTERONE RECEPTORS

Progesterone exerts its genomic effects through PR that belongs to the nuclear receptor family^{1,5,14,15}. These proteins act as transcription factors activated by their ligand that specifically regulate gene expression involved in metabolism, growth, and reproduction^{5,14,15}.

Progesterone crosses cell membranes by simple diffusion. Once in the nucleus or cytoplasm it binds to PR. This binding causes a structural change in the receptor that promotes the dissociation of heat shock proteins (HSP70, HSP90 and HSP65), thus exposing the region that allows phosphorylation and dimerization, resulting in a structure with high affinity to specific DNA sequences called hormone response elements. Once bound to hormone response elements, the complex ligand-receptor facilitates the assembly and stabilization of the

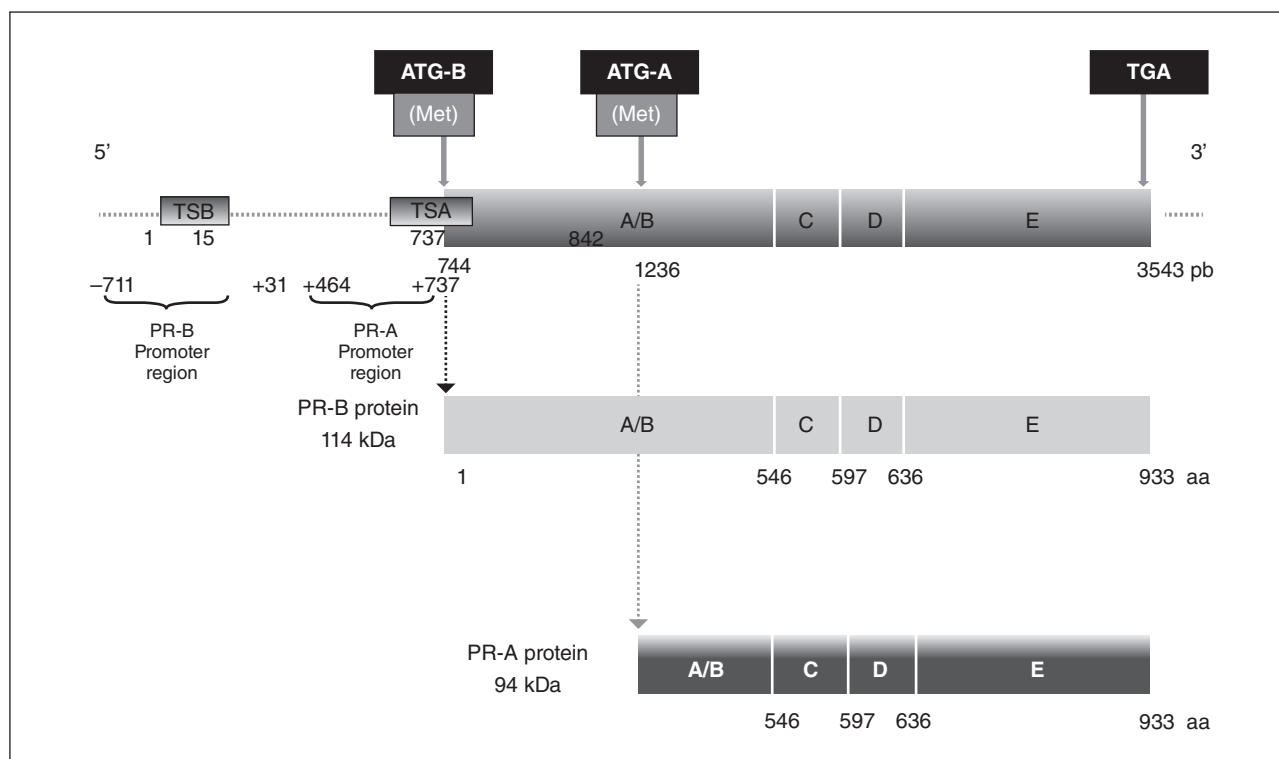


Figure 1. Representative scheme of gene and protein of progesterone receptor isoforms. TSB: transcription start site of progesterone receptor (PR)-B; TSA: transcription start site of the PR-A; ATG-B: translation start codon of PR-B; ATG-A: translation start codon of PR-A; TGA: translation stop codon of the PR; A-E: functional domains of the PR. Progesterone receptor consists of a number of different regions that are responsible for different functions of the receptor. The N-terminal part of PR contains two domains (A/B) and these domains contain activation function 1, which binds transcription factors responsible for the activation of the appropriate promoter for transcribing the isoforms. The activation function 1 is present in both PR-A and PR-B. A highly conserved DNA-binding domain (C) is located next to the A/B domain and consists of approximately 66-68 amino acids with two zinc finger structures. It is responsible for the interaction of the hormone-receptor complex with the hormone response element sequence (HRE), which is located within the promoter of the target gene. The hinge domain (D) stabilizes the receptor and contains the nuclear localization signal. A ligand-binding domain (E) is located at the carboxyl-terminal side of the D.

pre-initiation complex of transcription into the promoters of genes regulated by P4^{5,14-16}.

Progesterone receptor has two major isoforms called PR-B (112-120 kDa), and a truncated isoform called PR-A (80-94 kDa). Both PR isoforms are encoded by the same gene, but are transcribed from two different promoter sequences, the distal (from -711 to +31) corresponding to the promoter sequence of PR-B and the proximal (from 464 to 737) belonging to the promoter sequence of PR-A (Fig. 1). Broadly, PR-B is a stronger transcriptional activator than PR-A¹⁷. It has been shown that PR isoforms are functionally distinct in terms of their ability to activate transcription of target genes in the same cell type and regulate different physiological processes^{17,18}.

INDUCTION OF SEXUAL BEHAVIOR BY PROGESTERONE

The activity of different brain structures involved in the expression of female sexual behavior in the rat, such as hypothalamus and medial preoptic area, are modified by sex hormones, mainly E2 and P4^{3,14,19,20}.

It has been proposed that E2 acts initially through its intracellular receptor (ER), sensitizing neural substrates (e.g. ventromedial hypothalamus) related to the activation of female sexual behavior, while P4 triggers the expression of sexual behavior. If the female is ovariectomized (OVX) in the afternoon of proestrus, when the rat has reached the highest

levels of E2 but just before the P4 preovulatory peak, it will not display sexual behavior. Conversely, if P4 is administered at the time of OVX, the expression of lordosis behavior is facilitated. This fact is also observed if OVX is performed after P4 secretion has occurred^{14,21}.

Estrous behavior in rats can be induced by a single administration of 17 β -estradiol without subsequent injection of P4, although this occurs with less intensity. Progesterone injection significantly increases receptivity levels compared to those obtained with a single administration of E2. However, for the effectiveness of P4 administration in inducing lordosis behavior, females must have been exposed to estrogen for a minimum period of 12-18 hours²¹.

Moreover, the latency of lordosis behavior depends on the route of administration. For example, P4 administered subcutaneously facilitates receptivity between 2-6 hours²¹, whereas when is injected intravenously, lordosis may begin in around 30 minutes in 75% of the animals²². It has been reported that initiation of lordosis can be observed one hour after P4 implantation as crystals in some brain areas. However, other authors have observed a significant increase until 4-5 hours after brain implant²³.

PROGESTERONE RECEPTOR EXPRESSION IN SEXUAL BEHAVIOR

Estradiol induces expression of PR in brain areas such as the ventromedial hypothalamus and the medial preoptic area. These regions are related to the induction of sexual behavior in rodents^{14,19}. Considerable research has been developed to study the temporal correlation between the induction of PR by estrogens and the display of such behavior after treatment with P4.

Experiments in OVX rats showed that the maximum levels in facilitating sexual behavior by P4 and induction of PR in the hypothalamus were obtained 24 and 48 hours after treatment with E2. The beginning and the disappearance of estrous behavior induced by P4 in rats pretreated with E2 was explored, and it concurred with the increase and

decrease of PR in the ventromedial hypothalamus and medial preoptic area¹⁹.

In a study in guinea pigs, it was found that PR expression in the ventromedial hypothalamus and medial preoptic area precedes the onset of mating behavior induced by P4 in females previously treated with E2. A significant reduction in PR was also found in the same brain areas during the refractory period of sexual behavior (sequential inhibition)^{14,19}.

In transgenic mice with a disruption in the PR gene (PRKO mice), it has been observed that these animals are infertile, have deficiencies in the display of sexual behavior, present developmental malformations in the mammary glands²⁴ and changes in circulating concentrations of follicle-stimulating hormone, luteinizing hormone, and prolactin^{25,26}, although the effects of overexpression of any of PR isoforms on sexual behavior are unknown.

To assess the contribution of individual isoforms in reproductive behavior, different methodological strategies have been addressed. One of them is the use of specific mutants for PR-A or PR-B isoforms. This has been achieved by introducing a point mutation in the PR gene at codons for methionine 1 and methionine 166, which are the sites of initiation of translation for PR-B and PR-A isoforms, respectively²⁷.

In transgenic mice that express only one PR isoform, the role of each isoform in modulating sexual receptivity has been determined. In this analysis, it was observed that PR-A isoform plays a key role in facilitating the receptive sexual behavior. Both isoforms are essential for the display of dopamine-mediated sexual behavior, while PR-A plays an important role in sexual behavior modulated by cyclic adenosine monophosphate (cAMP)^{14, 28}.

In another study it was reported that RU486, an antagonist of PR, inhibited lordosis induced by P4, and the administration of PR oligonucleotide antisense (which interferes with protein synthesis) in the ventromedial hypothalamus of OVX females also inhibited estrus response, including proceptive behavior induced by P4 and a decrease in PR expression^{29,30}.

Another experimental strategy that has been used to elucidate the role of each PR isoform in the deployment of sexual behavior is the use of antisense

oligonucleotides specific for each isoform. We have reported that intracerebroventricular administration of antisense oligonucleotides to PR-B and total PR (PR-A + PR-B) in adult female rats treated with E2 and P4, as well as E2 and two progesterone metabolites: 5α dihydroprogesterone (DHP) and $5\beta,3\beta$ -pregnan-20-one ($5\beta,3\beta$ -Pgl), inhibited lordosis behavior. In the case of animals treated with $5\beta,3\beta$ -Pgl, PR-B antisense oligonucleotide inhibited lordosis similar to total PR antisense oligonucleotide. These results indicate that PR-B isoform is essential for the display of female sexual behavior in rats³¹.

SEQUENTIAL INHIBITION, PROGESTERONE RECEPTOR AND PROTEASOME

After females of different species of rodents have displayed sexual behavior, there is a period of time without response to mating^{19,30}. This inhibition in sexual response attributed to P4 was first described in guinea pigs and later in rats¹⁹. Thus, P4 has a dual effect on female sexual behavior: initially it stimulates, but after a few hours this same hormone inhibits sexual behavior.

The process by which P4 causes inhibition of estrus behavior in rodents is known as sequential inhibition and occurs after P4 has facilitated the estrus behavior in OVX animals previously treated with E2. This facilitation is followed by a period in which females are refractory to a second administration of P4. The level of sequential inhibition dependent of P4 dose was initially observed by Boiling and Blandau¹⁹ who reported that guinea pigs entering estrus by sequential administration of E2 and P4 did not show this behavior again after a second injection of P4. Similar results have been obtained in rat and hamster, even when given a second series of E2 + P4 a day after the animals had been receptive^{10,19,32}. These data show that after sequential treatment of E2 and P4, the female requires a period of time to recover before it can respond again to hormone treatment.

In OVX rats initially treated with E2 and later with P4, the subcutaneous administration of PSI (N-benzyloxy-carbonyl-Ile-Glu(Ot-butyl)-Ala-Leu-al), an inhibitor of

the proteasome, significantly increased the content of PR in the preoptic area and hippocampus. The increase in the content of PR-A and PR-B after the administration of PSI in the preoptic area was 90%, while in the hippocampus it was 50%. Very interestingly, PSI did not change the content of PR in the cerebral cortex³³, suggesting that the 26S proteasome participates in the *in vivo* tissue-specific turnover of PR in the central nervous system of the rat^{14,33}.

Degradation of PR by the 26S proteasome has important consequences in the regulation of sexual behavior in female rats. Our group demonstrated that systemic administration of two 26S PSI, a reversible protease inhibitor with chymotrypsin activity, and TLCK, an irreversible inhibitor of serine protease, blocked the sequential inhibition of sexual behavior in both proceptive and lordosis components of OVX female rats. This effect is correlated with an increase in the content of both PR isoforms in the hypothalamus and the preoptic area, thus suggesting that the behavioral insensitivity to P4 during sequential inhibition is due to degradation of PR by the 26S proteasome induced by its natural ligand¹⁹.

EFFECT OF PROGESTERONE METABOLITES IN SEXUAL BEHAVIOR

Progesterone is rapidly metabolized in the brain in a variety of reduced progestins on ring A^{10,34}. For example, P4 is converted to 5α -DHP by the action of 5α -reductase, and by a further reduction at carbon 3, 5α -DHP is transformed into 5α - 3α -tetrahydroprogesterone ($5\alpha,3\alpha$ -THP)³⁵.

In female rats previously treated with estrogens, systemic injection of 5α -DHP and $5\alpha,3\alpha$ -Pgl, as well as P4, induces intense sexual behavior (lordosis and proceptive behavior)^{30,36}. Furthermore, when injected intravenously and intracerebrally, the 5α -DHP and $5\alpha,3\alpha$ -Pgl stimulate estrus behavior more strongly than P4^{3,37}.

The cellular mechanism underlying sexual behavior induced by progestins reduced in the A ring is not well defined. One possibility is that the PR is a common molecular effector that modulates the

enabling actions of progestins in lordosis. This idea is supported by the ability of RU486 (PR antagonist) to attenuate the behavioral effects of these progestins when administered systemically^{22,38}. However, some of these progestins, especially those with the 3 α configuration, are potent modulators of GABA_A^{22,39} and glutamate^{40,41} receptors.

It is possible that lordosis facilitation produced by these progestins was partially mediated through membrane effects, especially in the midbrain^{3,30,39,42}.

The DHP can have two conversions. First, it can be reduced to 3 α , 5 α -tetrahydroprogesterone (allopregnenolone or THP) by the 3 α -hydroxy steroids (3 α -HSD) oxide reductase, or it can be converted to 3 β , 5 α -tetrahydroprogesterone (3 β , 5 α -THP) when the DHP is the substrate of 3 β -hydroxy steroids (3 β -HSD) oxide reductase^{5,10,43}. Moreover, the reaction between the progesterone metabolites, DHP and THP, is reversible. The THP may be oxidized to DHP in a reaction catalyzed by an enzyme isoform 3 α -HSD that is attached to the membrane^{5,10,44}.

The THP interacts with the benzodiazepine site of the GABA_A receptor^{15,45,46}. Although it has no effect on the activity of the PR⁴⁷, THP is an effective modulator of the GABA receptor in non-murine rodents^{15,45,48}. In addition, THP facilitates responsiveness when applied in the ventromedial hypothalamus of the rat, hamster, and mouse^{3,49,50}.

INTERCOMMUNICATION BETWEEN GENOMIC AND NON-GENOMIC EVENTS AND PROGESTERONE RECEPTOR FOR REGULATION OF FEMALE SEXUAL BEHAVIOR

The estrous behavior in rodents is triggered by the binding of intracellular P4 to PR. Many pharmacological studies initiated in the early 1970s showed that non-steroidal agents, in principle GnRH⁵¹ and prostaglandin E2 (PGE2)⁵², may facilitate lordosis behavior in OVX rats treated with E2. Years later, the number of non-steroidal drugs able to stimulate lordosis in this model has significantly increased. These include agents with

several structures: small peptides (oxytocin, GnRH, melanocyte-stimulating hormone); proteins (prolactin, adrenocorticotrophic hormone); neurotransmitters (norepinephrine, dopamine, GABA, acetylcholine) and prostaglandins^{14,30,53}.

The diversity of chemical structures of these lordogenic agents obviously excludes the possibility of having a common action in a single receptor. Moreover, none of these agents has affinity for ER or PR. However, it is proposed that lordosis behavior is the result of the convergence of signals (crosstalk) between sex hormones and other chemical messengers such as GnRH, PGE2, and dopamine, among others. These agents may replace P4, although less efficiently, in the induction of female sexual behavior in OVX rats previously treated with E2^{22,28}. For a time it was thought that this event implied the existence of several mechanisms involved for each of these compounds in the induction of lordosis. Recent results have shown that lordogenic action of these agents occurs through a common mediator: the PR. Thus, the administration of the anti-progestin RU486, which binds to PR, blocks the stimulatory action on lordosis induced by GnRH, PGE2, and cAMP²². However, other proteins associated to PR can participate in the integration of the expression of estrus behavior. These activating agents also stimulate other transcription factors that can interact with processes regulated by PR responsive elements through various signaling pathways. For example, CREB (phosphoprotein that binds to elements responsive to cAMP [CRE] in the DNA sequence) is an interesting candidate, because is activated by kinases (A, C and calcium/calmodulin) involved in estrus behavior in rodents.

Several findings indicate that these compounds induce phosphorylation by activating PR second messenger-dependent kinases. Phosphorylation of specific sites of PR is coupled to multiple functions thereof, including nuclear location in response to activation of mitogen-activated protein kinase (MAPK), transcriptional synergism in the presence of P4, and downregulation by the 26S ubiquitin-proteasome pathway^{14,54}.

The importance of PR phosphorylation induced by various agents coincides with the observation that intracerebral administration of cAMP or forskolin

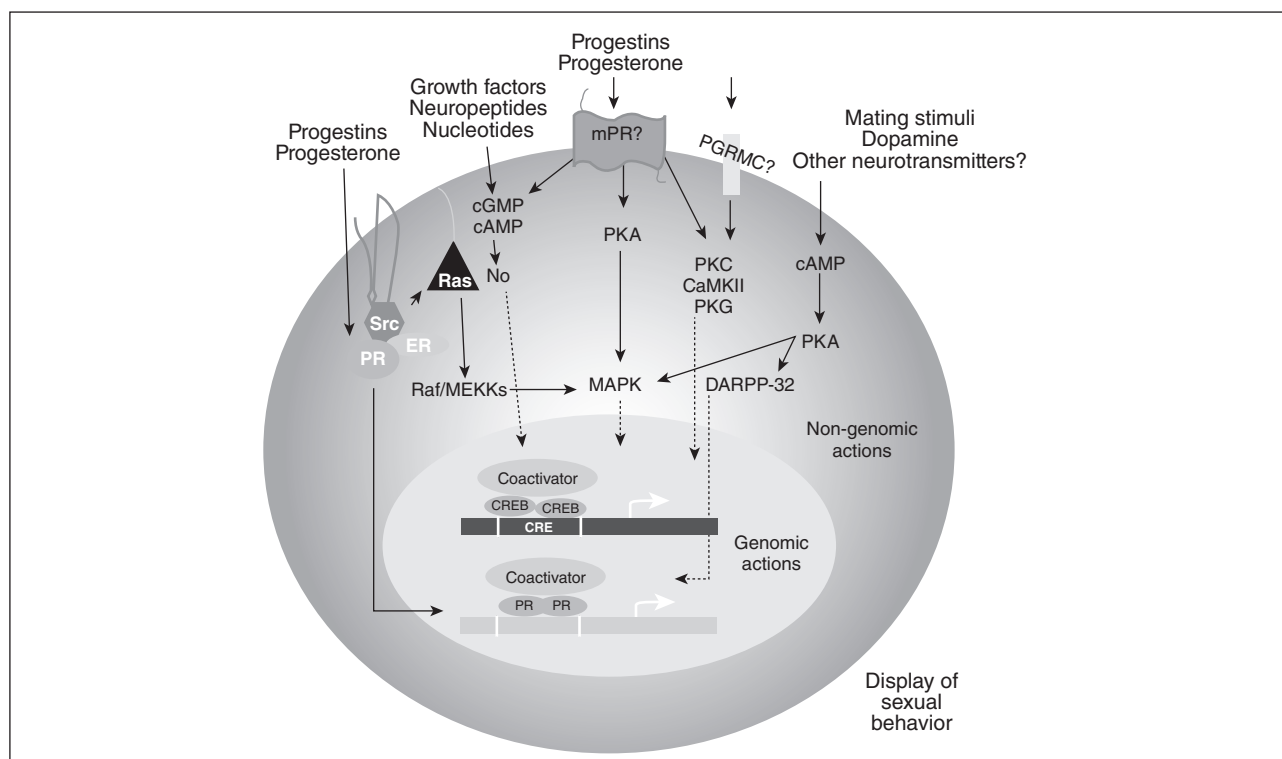


Figure 2. Representation of the interaction between the genomic and non-genomic pathways in the female reproductive behavior in rodents. Genomic actions of progesterone (P), mediated by intracellular progesterone receptor (PR) functioning as transcription factor, remain the primary mechanism of P action in female sex behavior. Non-genomic activation of cytoplasmic signaling pathways mediated by kinases, whether initiated by nonsteroidal agents or by progestins, can affect both transcription-dependent and -independent actions. Several agents influence female sexual behavior by activating extranuclear protein kinases A and C (PKA, PKC), calcium and calmodulin kinase II (CaMKII), and mitogen-activated protein kinase (MAPK). Progesterone acting via the extranuclear PKA/MAPK/DARPP-32 pathway can cause a decrease in phosphatase activity and an increase in phosphorylation of PR and/or its coactivators. Mating stimuli (VCS), dopamine or other neurotransmitters can stimulate PKA activation, phosphorylates DARPP-32, leading to the activation of CREB/PR/coactivators. The VCS-stimulated PKA activation can also interact with MAPK cascade. Neuropeptides, nucleotides, growth factors, GnRH, and PGE 2 can act through various receptor-and/or second messengers (cAMP, cGMP, NO) and transmit signals to the nuclear PR or other transcription factors. Progesterone also exhibits short-latency effects via modulation of putative cell surface PR receptors, ion channels, and mechanisms coupled to cytoplasmic second messenger signaling cascades, independent of gene transcription. These non-genomic effects are triggered at the membrane surface, the identification of two types of novel membrane proteins unrelated to classical PR, membrane PR (mPR) and progesterone receptor membrane component 1 (PGRMC1) in the brain, the functional role of these putative membrane receptors remains to be determined. Interactions between multiple pathways may serve as an amplification mechanism to converge on nuclear transcription factors and/or coactivators to regulate gene transcription and translation, to facilitate female sex behavior (*adapted from Mani and Blaustein 2012*³⁰).

(an adenylate cyclase activator) facilitates the lordosis behavior in rats pretreated with E2. In addition, the role of cAMP in the activation of estrus behavior by P4 was supported by the findings where phosphodiesterase inhibitors, such as theophylline, significantly increased the effects of various doses of P4 on the lordosis behavior of rats pretreated with E2⁵³. Studies in our laboratory have shown the involvement of other second messenger systems related to the expression of female sexual behavior in rats, such as the nitric oxide-cGMP-kinase G system¹⁹.

The signal convergence between nonsteroidal agents and steroid hormone receptors has been shown for PR as well as for ER. In this case, trophic factors such as nerve growth factor and epidermal growth factor synergize the effects of estrogen at an ER level, causing various estrogen-dependent responses.

Progesterone actions in the ventral tegmental area influence the intensity and duration of sexual receptivity of rodents by non-classical and quick actions performed by this hormone⁵⁵. Free P4 and

P4 attached to large molecules such as BSA and HRP, which are impermeable to the cell membrane, when applied in the ventral tegmental area rapidly increase lordosis, suggesting that P4 does not broadcast through the cell membrane to perform their functions in that region. Also, if P4 is directly administered into the medial tegmental area, it facilitates lordosis in five minutes. This effect is estimated to occur in a short time, thus it is considered that the actions of P4 in this region may occur through membrane receptors to neurotransmitters such as GABA, dopamine, glutamate, or membrane PR (Fig. 2). The membrane PR β has a very important function in facilitation of lordosis in rodents. The decrease in membrane PR β expression in midbrain diminishes lordosis in rats³.

PROSPECTS

Several studies have demonstrated the importance of the activation of PR in the regulation of female sexual behavior in rodents. The activation mechanisms of PR may be ligand-dependent or independent. The characterization of PR isoforms has expanded and changed the landscape of mechanisms of action of P4 on female sexual behavior in rodents; however, there are different aspects that need further study. These will allow determining the role of P4 and its receptors in regulating this behavior. It will be essential to understand how neuronal kinases and phosphatases activated by neurotransmitters regulate the balance between PR transcriptional active and inactive states and its co-regulators in various brain regions, which are involved in the regulation of female sexual behavior. It is also important to elucidate the molecular mechanisms by which this balance can be regulated, with second and third messengers acting as signal amplifiers.

Future research will probably demonstrate the mechanisms in which multiple signals converge and reinforce the neural response to environment and behavioral events that alter the effects of steroid hormones in female sexual behavior in rodents.

ACKNOWLEDGMENTS

We thank Julia Segura from Escuela Superior de Medicina IPN for the revision of the English manuscript.

REFERENCES

- Guerra-Araiza C, Camacho-Arroyo I. Progesterone receptor isoforms: function and regulation. *Rev Invest Clin*. 2000;52:686-91.
- Takimoto GS, Tung L, Abdel-Hafiz H, et al. Functional properties of the N-terminal region of progesterone receptors and their mechanistic relationship to structure. *J Steroid Biochem Mol Biol*. 2003;85:209-19.
- Frye CA, Walf AA, Kohtz AS, Zhu Y. Membrane progesterone receptors in the midbrain ventral tegmental area are required for progesterone-facilitated lordosis of rats. *Horm Behav*. 2013;64:539-45.
- Camacho-Arroyo I, Pasapera AM, Pérez-Palacios G, Cerbón MA. Progesterone and its metabolites in central nervous system function. *Rev Invest Clin*. 1995;47:329-40.
- Brinton RD, Thompson RF, Foy MR, et al. Progesterone receptors form and function in brain. *Front Neuroendocrinol*. 2008;29:313-39.
- Camacho-Arroyo I. Progesterone receptor isoforms expression and progesterone actions in the brain in "Life Sciences". S.G. Pandalai (Ed). *Research Signpost Kerala India*. 2003;221-42.
- Caldwell JD, Suleman F, Chou SH, Shapiro RA, Herbert Z, Jirikowski GF. Emerging roles of steroid-binding globulins. *Horm Metab Res*. 2006;38:206-18.
- Camacho-Arroyo I, González-Arenas A, González-Moran G. Ontogenic variations in the content and distribution of progesterone receptor isoforms in the reproductive tract and brain of chicks. *Comp Biochem Physiol A Mol Integr Physiol*. 2007;146:644-52.
- Baudry M, Bi X, Aguirre C. Progesterone-estrogen interactions in synaptic plasticity and neuroprotection. *Neurosci*. 2013;239:280-94.
- Schumacher M, Mattern C, Ghoumari A, et al. Revisiting the role of progesterone and allopregnanolone in the nervous system: Resurgence of the progesterone receptors. *Prog Neurobiol*. 2014;113:6-39.
- Belot MP, Abdennebi-Najar L, Gaudin F, et al. Progesterone reduces the migration of mast cells toward the chemokine stromal cell-derived factor-1/CXCL12 with an accompanying decrease in CXCR4 receptors. *Am J Physiol Endocrinol Metab*. 2007;292:E1410-7.
- Westwood FR. The female rat reproductive cycle: a practical histological guide to staging. *Toxicol Pathol*. 2008;3:375-84.
- González-Mariscal G, González-Flores O. Conducta sexual femenina en Motivación y conducta. Escobar-Briones C y Aguilar-Roblero RA (Eds.). *Manual Moderno, México D.F.* 2002;277-97.
- Mani S, Portillo W. Activation of progesterone receptors in female reproductive behavior: Interactions with neurotransmitters. *Front Neuroendocrinol*. 2010;31:157-71.
- Melcangi RC, Giatti S, Calabrese D, et al. Levels and actions of progesterone and its metabolites in the nervous system during physiological and pathological conditions. *Prog Neurobiol*. 2014;113:56-69.
- Camacho-Arroyo I, Rodríguez-Dorantes M. Transcriptional activity regulated by progesterone receptor isoforms in "Molecular Endocrinology" P Joseph-Bravo (Ed). *Research Signpost, Kerala India*, 2006;25-38.
- Jacobsen BM, Horwitz KB. Progesterone receptors, their isoforms and progesterone regulated transcription. *Mol Cell Endocrinol*. 2012;357:18-29.
- Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM, Horwitz KB. Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. *J Biol Chem*. 2002;277:5209-18.
- González-Flores O, Shu J, Camacho-Arroyo I, Etgen AM. Regulation of lordosis by cyclic 3',5'-guanosine monophosphate, progesterone, and its 5 α -reduced metabolites involves mitogen-activated protein kinase. *Endocrinology*. 2004;145:5560-7.
- Frye CA, Paris JJ, Walf AA, Rusconi JC. Effects and mechanisms of 3 α ,5 α -THP on emotion, motivation, and reward functions involving pregnane xenobiotic receptor. *Front Neurosci*. 2012;19:5-136.
- Blaustein JD. Neuroendocrine regulation of feminine sexual behavior: lessons from rodent models and thoughts about humans. *Annu Rev Psychol*. 2008;59:93-118.

22. Beyer C, González-Flores O, González-Mariscal G, Ring A. reduced progestins potentially stimulate estrus behavior in rats: paradoxical effect through the progesterone receptor. *Physiol Behav.* 1995; 58:985-93.
23. Barfield RJ, Glaser JH, Rubin BS, Etgen AM. Behavioral effects of progestin in the brain. *Psychoneuroendocrinology.* 1984;9:217-31.
24. Conneely OM, Mulac-Jericevic B, Lydon JP. Progesterone-dependent regulation of female reproductive activity by two distinct progesterone receptor isoforms. *Steroids.* 2003;68:771-8.
25. Chappell PE, Lydon JP, Conneely OM, O'Malley BW, Levine JE. Endocrine defects in mice carrying a null mutation for the progesterone receptor gene. *Endocrinology.* 1997;138:4147-52.
26. Petersen SL, Intlekofer KA, Moura-Conlon PJ, Brewer DN, Del Pino Sans J, Lopez JA. Novel progesterone receptors: neural localization and possible functions. *Front Neurosci.* 2013;9:7-164.
27. Mulac-Jericevic B, Lydon JP, DeMayo FJ, Conneely OM. Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. *Proc Natl Acad Sci USA.* 2003;100:9744-9.
28. Mani SK. Signaling mechanisms in progesterone-neurotransmitter interactions. *Neurosci.* 2006;138:773-81.
29. Pollio G, Xue P, Zanisi M, Nicolin A, Maggi A. Antisense oligonucleotide blocks progesterone-induced lordosis behavior in ovariectomized rats. *Brain Res Mol Brain Res.* 1993;19:135-9.
30. Mani SK, Blaustein JD. Neural progestin receptors and female sexual behavior. *Neuroendocrinology.* 2012;96:152-61.
31. Guerra-Araiza C, Gomora-Arrati P, García-Juárez M, et al. role of progesterone receptor isoforms in female sexual behavior induced by progestins in rats. *Neuroendocrinology.* 2009;90:73-81.
32. Mani SK, Anselmo-Franci J, Lara H, et al. Neuroendocrine regulation and homeostasis. *J Neuroendocrinol.* 2014;26:555-6.
33. Camacho-Arroyo I, Villamar-Cruz O, González-Arenas A, Guerra-Araiza C. Participation of the 26S proteasome in the regulation of progesterone receptor concentrations in the rat brain. *Neuroendocrinology.* 2002; 76:267-71.
34. Poletti A, Martini L. Androgen-activating enzymes in the central nervous system. *J Steroid Biochem Mol Biol.* 1999;69:117-22.
35. Griffin LD, Mellon SH. Biosynthesis of the neurosteroid 3 alpha-hydroxy-4-pregnen-20-one (3 alpha hp), a specific inhibitor of FSH release. *Endocrinology.* 2001;142:4617-22.
36. Gorzalka BB, Whalen RE. The effects of progestins, mineralocorticoids, glucocorticoids, and steroid solubility on the induction of sexual receptivity in rats. *Horm Behav.* 1977;8:94-9.
37. Frye CA, Bayon LE, Pursnani NK, Purdy RH. The neurosteroids, progesterone and 3alpha,5alpha-THP, enhance sexual motivation, receptivity, and proceptivity in female rats. *Brain Res.* 1998;808:72-83.
38. Gómora-Arrati P, Beyer C, Lima-Hernández FJ, Gracia ME, Etgen AM, González-Flores O. GnRH mediates estrous behavior induced by ring A reduced progestins and vaginocervical stimulation. *Behav Brain Res.* 2008;187:1-8.
39. Frye CA. Novel substrates for, and sources of, progestogens for reproduction. *J Neuroendocrinol.* 2011;23:961-73.
40. Prince RJ, Simmonds MA. Steroid modulation of the strychnine-sensitive glycine receptor. *Neuropharmacology.* 1992;31:201-5.
41. Frye CA, Walf AA, Petralia SM. Progestins' effects on sexual behavior of female rats and hamsters involving D1 and GABA(A) receptors in the ventral tegmental area may be G-protein-dependent. *Behav Brain Res.* 2006;172:286-93.
42. Delville Y. Progesterone-facilitated sexual receptivity: a review of arguments supporting a nongenomic mechanism. *Neurosci Biobehav Rev.* 1991;15:407-14.
43. Compagnone NA, Mellon SH. Neurosteroids: biosynthesis and function of these novel neuromodulators. *Front Neuroendocrinol.* 2000;21:1-56.
44. Jung-Testas I, Hu ZY, Baulieu EE, Robel P. Neurosteroids: biosynthesis of pregnenolone and progesterone in primary cultures of rat glial cells. *Endocrinology.* 1989;125:2083-91.
45. Belelli D, Lambert JJ. Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci.* 2005;6:565-75.
46. Baulieu EE, Robel P. Non-genomic mechanisms of action of steroid hormones. *Ciba Found Symp.* 1995;191:24-37; discussion: 37-42.
47. Iswari S, Colas AE, Karavolas HJ. Binding of 5 alpha-dihydroprogesterone and other progestins to female rat anterior pituitary nuclear extracts. *Steroids.* 1986;47:189-203.
48. Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. *Science.* 1986;232:1004-7.
49. Frye CA, Vongher JM. Progesterone and 3alpha,5alpha-THP enhance sexual receptivity in mice. *Behav Neurosci.* 2001;115:1118-28.
50. Frye CA, Paris JJ, Rhodes ME. Exploratory, anti-anxiety, social, and sexual behaviors of rats in behavioral estrus is attenuated with inhibition of 3alpha,5alpha-THP formation in the midbrain ventral tegmental area. *Behav Brain Res.* 2008;193:269-76.
51. Moss RL, McCann SM. Induction of mating behaviour in rats by luteinizing hormone-releasing factor. *Science.* 1973;181:177-9.
52. Rodríguez-Sierra JF, Komisaruk BR. Effects of prostaglandin E2 and indomethacin on sexual behaviour in the female rat. *Horm Behav.* 1977;9:281-9.
53. Beyer C, González-Mariscal G. Elevation in hypothalamic cyclic AMP as a common factor in the facilitation of lordosis in rodents: a working hypothesis. *Ann NY Acad Sci.* 1986;474:270-81.
54. Lange CA, Shen T, Horwitz KB. Phosphorylation of human progesterone receptors at serine-294 by mitogen-activated protein kinase signals their degradation by the 26S proteasome. *Proc Natl Acad Sci USA.* 2000;97:1032-7.
55. Frye CA. Steroids, reproductive endocrine function, and cognition: A review. *Minerva Ginecol.* 2009;6:563-85.