Progestin Receptors

Neuronal Integrators of Hormonal and Environmental Stimulation

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ABSTRACT: Although it originally was believed that neuronal steroid hormone receptors require binding to cognate ligand for activation, more recent evidence suggests that the receptors can be activated indirectly by other compounds, such as neurotransmitters and growth factors, acting through their own membrane receptors and specific intracellular signaling pathways. For example, as is the case with facilitation of sexual behavior by progesterone, facilitation of sexual behavior by D_1/D_5 dopamine receptor agonists is blocked by disruption of progestin receptors. Therefore, some dopamine agonists facilitate sexual behavior at least in part by a progestin receptor–dependent mechanism, as does progesterone. This "ligand-independent activation" of neuronal progestin receptors is not limited to dopamine agonists; a variety of other compounds, as well as mating stimulation, facilitate sexual receptivity by a progestin receptor–dependent process. Steroid hormone receptors also can be regulated by afferent input in another way. Various neurotransmitters upregulate or downregulate steroid hormone receptors in some neurons. This, in turn, presumably confers greater or decreased sensitivity to the particular factors that can activate the particular steroid receptor in those particular neurons. Therefore, steroid hormones are but one class of factors that can regulate and activate steroid hormone receptors. Some additional factors that activate steroid hormone receptors have been identified, as have some factors that can regulate concentrations of receptors. Relatively little is known at this time about the range of neurotransmitters, humoral factors, and intracellular signaling pathways that are involved.

KEYWORDS: progestin receptor; estrogen receptor; progesterone; estradiol; neural integration; hypothalamus; forebrain; sexual behavior; reproductive behavior; steroid hormones; ligand-independent activation

INTRODUCTION

Ovarian hormones have many effects in the brain which result in changes in behaviors and reproductive physiology.[1–3] A model that often is used to study the cellular mechanisms of ovarian hormones in the brain is the regulation of feminine

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sexual behavior by estradiol and progesterone. In this article, the role of neuronal progestin receptors in mediating the effects of progesterone on sexual behavior is discussed. Also, the influences of afferent input conveyed from the social environment via changes in neurotransmitter pathways on steroid hormone receptors are elaborated. We suggest that there is the potential for a great deal of regulation of progestin receptor–dependent processes in specific neurons by particular neurotransmitter pathways that convey environmental regulation of steroid hormone receptors.

Although the cellular processes of hormone action in the brain have been most extensively studied in rats, there is much commonality in the hormonal regulation of feminine sexual behavior in a variety of other rodent species, including guinea pigs, hamsters, and mice. During the estrous cycle,[4–8] the sequential secretion of estradiol and progesterone from the ovaries induces a period of sexual behavior around the time of ovulation. Ovariectomy eliminates the expression of feminine sexual behaviors by terminating the cyclic release of ovarian hormones.[4,9,10] Although ovariectomized rats, guinea pigs, and mice may respond to estradiol alone, sequential treatment with estradiol and progesterone is more effective,[9–11] and it makes the onset and termination of heat more predictable.[4,9,10] After heat terminates, the animals become transiently refractory to the effect of progesterone on sexual receptivity.[1,2,12–14] Because estradiol and progesterone have very predictable influences on feminine sexual behavior, the hormonal regulation of feminine sexual behavior provides a physiologically relevant model with which to investigate the cellular processes by which estradiol, progesterone, and other factors act on the brain to influence behavior and by which other afferent factors may influence neuronal function and behavior through these processes.

There is general agreement that steroid hormones act through steroid hormone receptors functioning as transcriptional regulators, as well as by other mechanisms. Steroid hormones diffuse freely into and out of all cells, but, in target cells containing receptors for the particular hormone, the hormones bind with high affinity to unoccupied receptors. Ligand binding to the receptor results in a conformational change, activation, and dimerization. Once bound to the chromatin, the hormone–receptor complex causes changes in gene expression,[15] leading to alterations in protein synthesis and consequently to changes in cellular function. Although some experiments suggested that the receptors are entirely or primarily nuclear proteins,[16,17] others suggested the presence of receptors in both cytoplasmic and nuclear locations, particularly in the brain.[18] However, the site of action of the receptors acting as transcriptional regulators is the cell nucleus, where they bind in concert with steroid receptor coregulators,[19,20] to steroid response elements on particular genes.

NEURAL PROGESTIN RECEPTORS AND BEHAVIOR

The dependence of progesterone-facilitated sexual behavior on progestin receptors in guinea pigs, rats, and mice has been shown by the use of progesterone antagonists,[21] antisense oligonucleotides to progestin receptor mRNA,[22–24] and progestin receptor gene disruption in mice.[25] Generally, when progestin receptor levels are elevated, animals respond to progesterone with the expression of sexual behavior, and when they are reduced, either by interference with the receptor, by lack of estradiol,

or by down-regulation by progesterone itself, animals are hyposensitive or unresponsive to progesterone.[1,2] Although there are numerous differences in the expression of sexual behaviors in different rodent species, rats, guinea pigs, and mice each require activation of neuronal progestin receptors for progesterone-facilitated sexual behavior.

LIGAND-INDEPENDENT ACTIVATION OF PROGESTIN RECEPTORS

Although it had been assumed that binding of receptors to their cognate ligands—the steroid hormones—was necessary for activation of the transcription factor receptors, it was shown more recently[26,27] that the COUP receptor, progestin receptor and estrogen receptor could each be activated *in vitro* by activation of D_1 dopamine receptors. The process by which dopamine or other compounds *indirectly* activate steroid hormone receptors through intracellular signaling pathways is referred to as *ligand-independent activation.*

A good deal of evidence now supports the idea that both progestin receptors and estrogen receptors can undergo ligand-independent activation. Most of this work, done *in vitro* and with peripheral tissues, has focused on activation of estrogen receptors by growth factors, such as epidermal growth factor[28] and insulin-like growth factor.[29] However, ligand-independent activation seems to be a common mechanism by which steroid hormone receptors are activated. Additional factors that can activate estrogen receptors and/or progestin receptors in various tissues by ligand-independent activation as well include GnRH,[30,31] D_1/D_5 dopaminergic agonists,[27] phorbol esters,[32] and cAMP,[29] MAP kinase,[33] and cyclins[34] (for review see Cenni and Picard[35]).

Despite the wealth of information concerning ligand-independent activation of steroid hormone receptors in nonneural tissues, relatively little work has been done on the idea that *neural* steroid hormone receptors can be activated by afferent influences. What is known is summarized here. Intracerebroventricular infusion of a D_1/D_5-specific dopamine receptor agonist substitutes for progesterone in facilitating sexual behavior in estradiol-primed rats.[36] As with progesterone activation of sexual behavior, progesterone antagonists, antisense oligonucleotides directed at the progestin receptor mRNA[22–24,37] or progestin receptor gene disruptions in mice[25] block this facilitation, supporting the idea that ligand-independent activation of progestin receptors mediates dopamine-facilitated feminine sexual behavior.

Ligand-independent activation of neuronal progestin receptors is not limited to activation by dopamine though. In fact, facilitation of sexual receptivity by a progestin receptor–dependent process has been observed for GnRH,[38,39] prostaglandin E2,[38] and nitric oxide.[40] Furthermore, stimulation of cAMP[38] and cGMP[41] each facilitate the expression of sexual behavior by a progestin receptor–dependent mechanism, implicating protein kinase A and protein kinase G in ligand-independent activation. Although the idea that many drugs that facilitate sexual behavior do so by influencing a common second messenger system was first proposed by Whalen and Lauber for cGMP[42] and by Beyer and Gonzalez-Mariscal for cAMP in 1986,[43] more recent data suggest that progestin receptors may be a component of a final common signaling pathway.

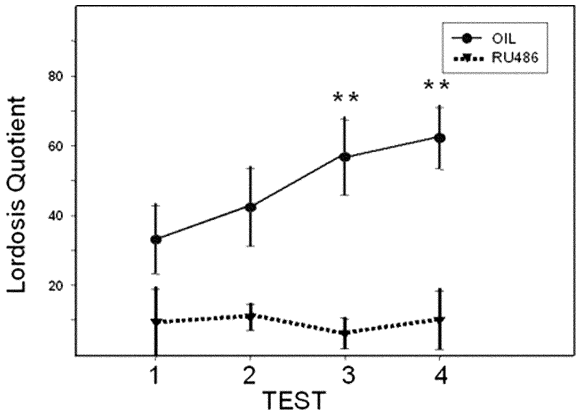

FIGURE 1. Effects of the progesterone antagonist RU486 or oil vehicle on sexual behavior of estradiol-primed female rats in response to repeated testing with a male rat. Each test is based on 15 min of mating with a male rat followed by 15 min without the male. Although rats were given vaginocervical stimulation with a plastic probe 15 min before the start of testing in this experiment, the additional stimulation is not essential for the enhancement of sexual behavior by mating. Data redrawn from Auger *et al.*[45]

Ligand-independent activation by afferent input appears to have an important role in the regulation of feminine sexual behavior by afferent input derived from mating stimulation in rats. When estradiol-treated, ovariectomized rats are repeatedly exposed to males, for example, for 15 min at a time followed by 15 min away for the male,[44] their lordosis increases over the course of a few hours, a response that is not dependent on the ovaries or adrenal glands.[45] Treatment with a progestin antagonist just before exposure to the males completely blocks this response (FIG. 1), although it does not block the rapid lordosis response to manual palpation *at the time of* vaginocervical stimulation.[45] This suggests that ligand-independent activation of progestin receptors is involved in the process by which environmental stimulation (i.e., mating-stimulation) enhances subsequent sexual behavior. Most importantly, these results also suggest that neuronal progestin receptors can undergo ligand-independent activation *in vivo* by a *physiologically relevant stimulus* in addition to pharmacologically by a D_1/D_5 dopamine agonist.

Ligand-independent activation of progestin receptors also is involved in the regulation of ovulation during the rat and mouse estrous cycle. Blockade of progestin receptors or inhibition of progestin receptor synthesis in the anteroventral periventricular area blocks some of the effects of estradiol on GnRH regulation. This is believed to be caused by inhibition of activation of progestin receptors, which occurs as a neuronal consequence of estradiol action (see Levine *et al.*[46] for review).

In a study of a vaginocervical stimulation–induced neuronal response that is apparently mediated by ligand-independent activation of progestin receptors, we found that injection of a progesterone antagonist an hour before vaginocervical stimulation with a plastic probe inhibits stimulation-induced Fos expression (a protein marker for a neuronal response to the stimulation) in the rostral medial preoptic

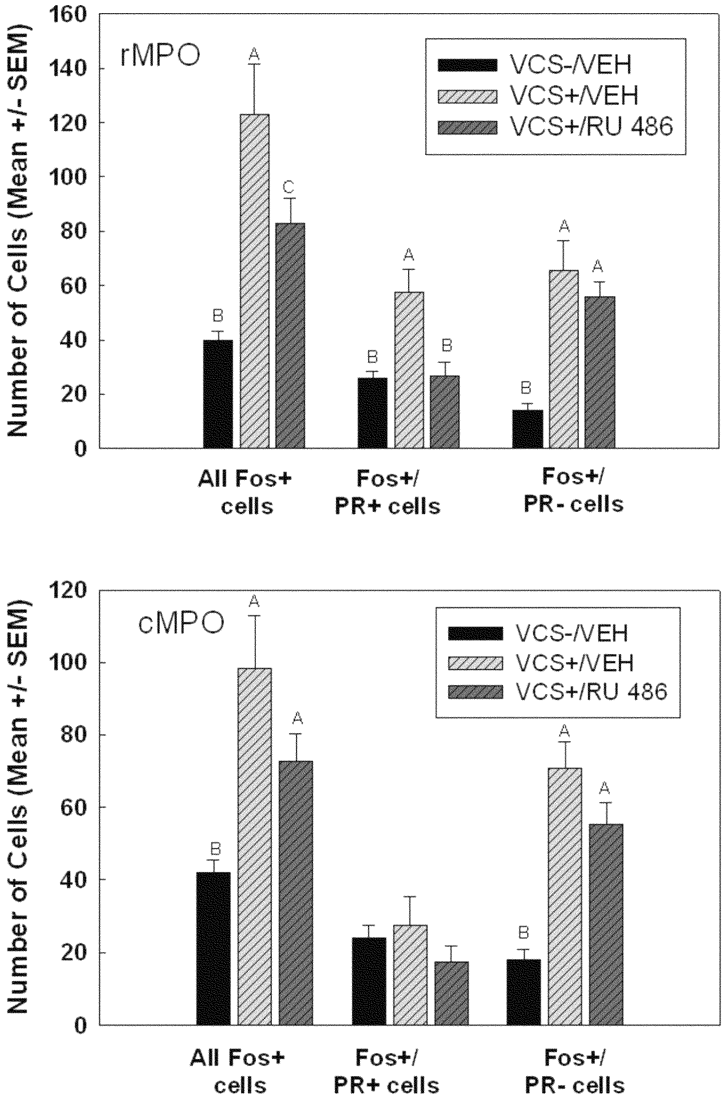

FIGURE 2. Number of cells expressing Fos-ir (all Fos+ cells), Fos-ir, and PR-ir (Fos+/PR+ cells) or Fos+ without PR-ir (Fos+/PR– cells) in the rostral medial preoptic area (rMPO) or caudal medial preoptic area (cMPO) after control stimulation (VCS–) preceded by vehicle (VCS–/VEH), vaginocervical stimulation preceded by vehicle (VCS+/VEH) or vaginocervical stimulation preceded by RU486 (VCS+/RU486). Bars with different letters over them denote statistically significant differences between groups. Data redrawn from Blaustein and Greco.[47]

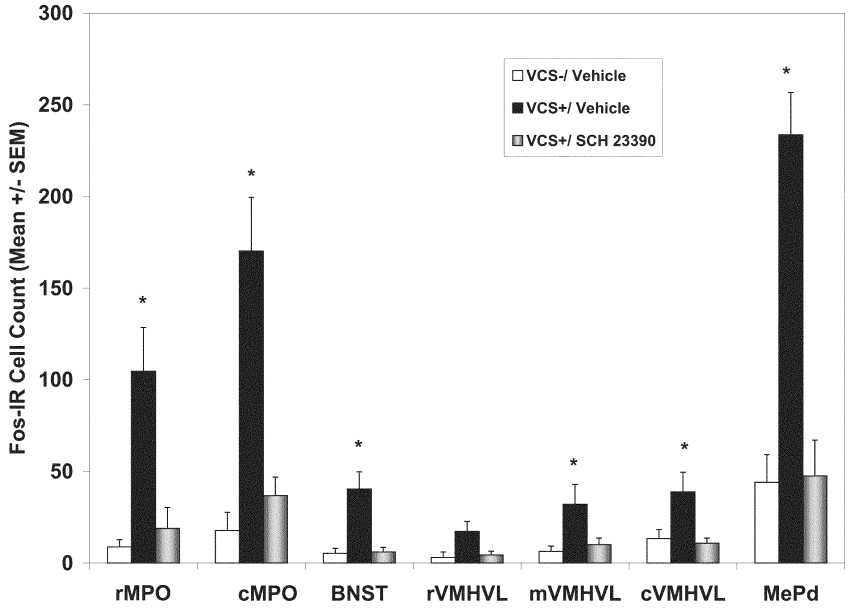

FIGURE 3. Numbers of Fos-ir cells (mean ± SEM) in forebrain areas after control stimulation (VCS–) or vaginocervical stimulation (VCS+) preceded by an injection of either saline or SCH 23390. Asterisks above bars indicate statistically significant difference between VCS + and other groups. rMPO, cMPO, rostral, caudal medial preoptic area; BNST, bed nucleus of stria terminalis; rVMHVL, mVMHVL, cVMHVL, rostral, mid, and caudal ventrolateral aspect of ventromedial hypothalamus; MePd, posterodorsal medial amygdala. Data redrawn from Quysner and Blaustein.[55]

area, bed nucleus of stria terminalis, and parts of the ventromedial hypothalamus, but not other areas studied.[45,47] Many of the neurons that responded with Fos expression coexpress progestin receptors.[48] In the rostral medial preoptic area, a progestin antagonist specifically blocked Fos expression in progestin receptor–containing cells, not in progestin receptor–negative cells (FIG. 2).[47] In contrast, in the caudal medial preoptic area, vaginocervical stimulation induced Fos expression only in progestin receptor–negative neurons, and the progestin antagonist was without effect. These results are consistent with the idea that, although Fos can be induced by mating stimulation in both neurons expressing and neurons not expressing progestin receptors, in those containing progestin receptors, the response to afferent input is *gated* by the progestin receptor. Furthermore, although not necessarily the *only* site involved, the data show that the rostral medial preoptic area is likely to be *one* of the sites at which genital stimulation can activate progestin receptors by the ligand-independent mechanism.

Consistent with the hypothesis that dopamine is one of the critical factors that is released in response to mating-related stimulation, resulting in ligand-independent activation of progestin receptors, mating-related stimuli induce the release of

dopamine,[49–52] as well as norepinephrine,[53,54] in the forebrain under a variety of circumstrances. These neurotransmitters then act on their receptors in specific neurons, which activate intracellular second messendger signaling pathways. Vaginocervical stimulation-induced Fos is completely blocked by a D_1/D_5 dopamine receptor antagonist in all forebrain areas studied (FIG. 3),[55] suggesting that dopamine is released in response to the vaginocervical stimulation which then stimulates D_1/D_5 dopamine receptors, which, in turn, induce a neuronal response (Fos expression). Preliminary immunocytochemical data suggest that D_5 dopamine receptors are coexpressed with progestin receptors in the ventromedial hypothalamus (J. C. Turcotte and J. D. Blaustein, unpublished material), suggesting a neuronal substrate by which dopaminergic neurotransmission might activate progestin receptors in some neurons.

Recent evidence suggests the importance of the phosphatase-1 inhibitor, DARPP-32,[56] in the process by which sexual behavior is induced by either progesterone or ligand-independent activation.[57] Activation of D_1/D_5 dopamine receptors induces an increase in the level of intracellular cAMP, which, in turn, induces phosphorylation on the Thr_{32} residue of DARPP-32, an inhibitor of protein phosphatase-1. Behavioral response to both progesterone and a D_1 dopamine receptor agonist, but not to serotonin, is blocked in DARPP-32 knockout mice, suggesting that DARPP-32 may be involved in phosphorylation and dephosphorylation of progestin receptors.[57] Because of this finding and the fact that vaginocervical stimulation induces the phosphorylation of DARPP-32 in some forebrain neurons, including progestin receptor–rich areas,[58] we suggested that one mechanism by which vaginocervical stimulation influences progestin receptor–dependent neuronal and behavioral changes is by activation of the DARPP-32 pathway. This, in turn, may lead to increased phosphorylation of progestin receptors or its coactivators resulting in activation of progestin receptors. Although much of the evidence is consistent with mating stimulation activating progestin receptors and facilitating sexual receptivity *via* a dopaminergic pathway, note that DARPP-32 can be phosphorylated by a variety of other neurotransmitters that act through either a protein kinase A or protein kinase G pathway, as well as by other intracellular signaling pathways.[56]

Steroid receptors can be activated by circulating hormones, so why might receptors in some neurons also be activated by afferent neurotransmitter input? We speculate that the teleological raison d'être for ligand-independent activation of neuronal steroid hormone receptors is to enable nonhormonal factors (i.e., afferent stimulation in the form of neurotransmitter release) to *fine-tune* and, perhaps in some, cases *turn on* the expression of neuronal and behavioral responses. This is best seen in the example of mating stimulation enhancing sexual receptivity over the course of a few hours described earlier. Although purely speculative, the purpose of this may be to synchronize the onset of receptive behavior in the female with the presence of adequate stimulation by male rats. In other words, if a female rat emerges from her burrow before she is fully receptive,[59] her initial interactions with male rats may hasten the onset of full sexual receptivity earlier than the progesterone would have done so. Although relatively little is known about the mechanisms of reflex ovulation, perhaps mating-induced, ligand-independent activation of progestin receptors provides a mechanism by which males can induce reflex ovulation by a neuronal pathway similar to that proposed for spontaneous ovulation.[46]

The duration of the period of sexual receptivity is the result of a complex interplay between factors that enhance and those that inhibit the further display. Interest-

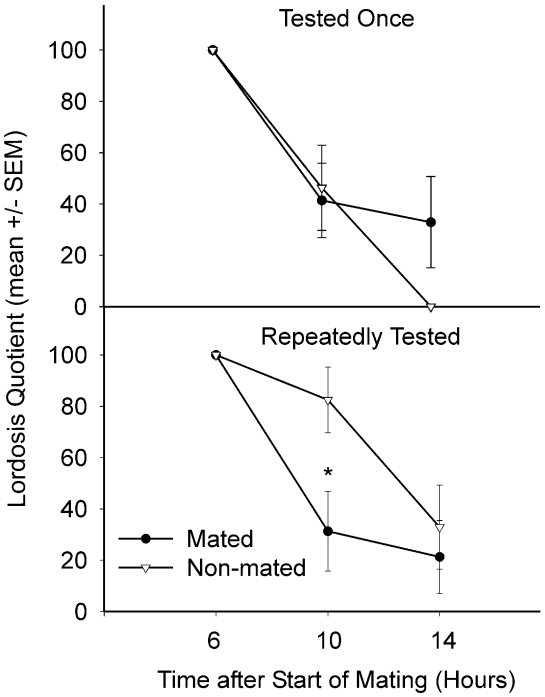

FIGURE 4. Lordosis quotients of female rats that had not been mated previously and those that received mounts and intromissions resulting in two ejaculations and were then tested 6, 10, and 14 h later (*top*). Lordosis quotients of female rats that had not been mated previously and those that received mounts and intromissions resulting in two ejaculations and then were tested at either 6, 10, or 14 h later (*bottom*). Contrast between the nonmated controls demonstrates that the testing at 6 and 10 h prolonged the duration of sexual receptivity. Prior mating exposure shortened heat duration only in the animals that were tested repeatedly, not in the animals tested only once. Data redrawn from reference Bennett *et al.*[60]

ingly, while mating stimulation in which intromissions were allowed abbreviated the period of sexual receptivity, mating stimulation in which intromissions were prevented somewhat delayed heat termination.[60] That is, although some aspects of mating stimulation shorten heat duration, others lengthen it (FIG. 4), and, as discussed earlier, some induce it. Some of this microregulation of sexual receptivity by the interactions of the female with a male might well involve acute regulation of activated progestin receptors by these stimuli from the social environment in concert with activation caused by hormonal changes. There is also one report of another signaling pathway involving epidermal growth factor activating neuronal estrogen receptors resulting in the expression of lordosis.[61] The possible contribution of ligand-independent activation of estrogen receptors also must be considered.

We also have suggested that a similar process could be involved in the induction or maintenance of maternal behavior by stimulation from pups, that is, the process

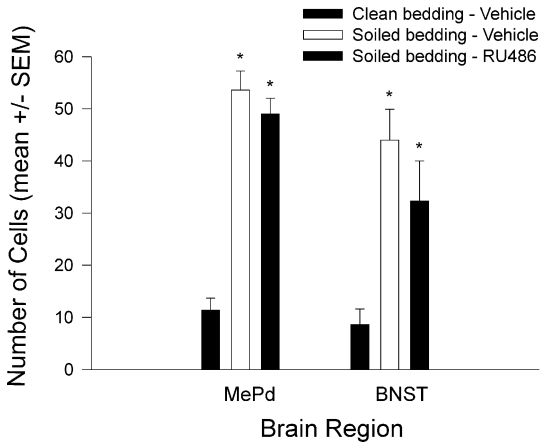

FIGURE 5. Mean number (±SEM) of Fos-immunoreactive cells in the posterodorsal medial amygdala (MePd) and bed nucleus of stria terminalis (BNST) after treatment with vehicle and exposure to clean bedding (Clean bedding - Vehicle), treatment with vehicle and exposure to male-soiled bedding (Soiled bedding - Vehicle), or treatment with RU486 and exposure to male-soiled bedding (Soiled bedding - RU486). *Significant difference from females treated with vehicle and exposure to clean bedding. Data redrawn from Bennett *et al.*[65]

by which rat pups induce maternal behavior in the absence of the typical hormonal changes associated with the onset of maternal behavior postpartum and also maintain maternal behavior in the absence of high estradiol levels. Perhaps one or more of the sensory stimuli associated with the pups results in the activation of estrogen receptors in critical neurons involved in the onset or maintenance of maternal behavior.[62]

AFFERENT REGULATION OF RECEPTOR CONCENTRATION

Not *all* influences of afferent input on steroid hormone-sensitive processes can be attributed to ligand-independent activation. Mating-induced enhancement of sexual behavior is also dependent on an accessory olfactory pathway.[44,63,64] However, unlike the case with vaginocervical stimulation, neurons in which Fos is induced after exposure to male-soiled bedding do not contain estradiol-induced progestin receptors.[65] Furthermore, unlike the case with vaginocervical stimulation-induced Fos expression, which is blocked by progesterone antagonists,[45] odor-induced Fos expression is not (FIG. 5).[65] Thus, the evidence suggests that, unlike vaginocervical stimulation, the effects of this particular olfactory pathway on sexual receptivity do not involve ligand-independent activation of progestin receptors.

The fact that odors do not seem to activate progestin receptors in the same way as genital stimulation does not imply that odors do not influence steroid receptors. First of all, although estrogen-induced progestin receptors were not coexpressed with Fos after exposure to odors from bedding soiled by male rats, ERα is coex-

pressed, leaving open the possibility that ligand-independent activation of ERα is involved. Furthermore, a second mode of interaction of afferent input with steroid hormone receptors is the regulation of steroid hormone receptor concentrations by neurotransmitters.[2] In fact, olfactory bulbectomy, which increases sensitivity to estradiol, increases the *concentration* of estrogen receptors in the medial amygdala.[66] An increase in receptor levels would be expected to alter *sensitivity* or *responsiveness* of particular neurons to estradiol or to other factors that can activate estrogen receptors. That is, a greater concentration of receptors in a particular neuron would increase the probability of activation by a particular ligand or other factor. Note that regulation of the concentrations of neuronal progestin, estrogen, and glucocorticoid receptors by an array of neurotransmitters and social environmental factors has been described (for review, see Blaustein and Erskine[2]).

SUMMARY

In summary, there are at least two modes of regulation of neuronal steroid hormone receptors by afferent input. Neurotransmitters and perhaps humoral factors as well can activate steroid hormone receptors independently of cognate hormone by ligand-independent activation. Ligand-independent activation of neuronal progestin receptors with consequent neuronal and behavioral changes can be induced not only by pharmacological means, but also by elements of the social environment as well. In addition, some neurotransmitters can, under some circumstances, regulate the concentrations of particular steroid hormone receptors in populations of neurons. This regulation of the concentration of receptors would be expected to alter the sensitivity of particular neurons to factors (including steroid hormones as well as neurotransmitters and other factors conveying input from the social environment) that can activate the receptors. Besides demonstrating novel mechanisms by which the social environment influences neuronal function and behavior, these modes of regulation of steroid receptors are relevant to other work in neuroendocrinology. It is possible that in some experiments in which the effects of neurotransmitters on progesterone-influenced neuronal and behavioral responses are assessed, the drugs may be inducing ligand-independent activation of progestin receptors with consequent progestin receptor–dependent changes in behavior and physiology. Alternatively, in other cases, drugs may influence the sensitivity of particular neurons to a particular steroid hormone by up- or down-regulation of steroid hormone receptors. A comprehensive understanding of the neuronal processes involved in the regulation of feminine sexual behavior requires an understanding of the identity of the afferent factors that influence these processes, as well as the cellular mechanisms by which this occurs.

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