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Minireview: Neuronal Steroid Hormone Receptors: They're Not Just for Hormones Anymore

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The ovarian steroid hormones have numerous effects on the brain, many of which are mediated, at least in part, by interaction with intracellular steroid hormone receptors acting as regulators of transcription. These intracellular steroid hormone receptors have often been considered to be activated solely by cognate hormone. However, during the past decade, numerous studies have shown that the receptors can be activated by neurotransmitters and intracellular signaling systems, through a process that does not require hormone. Although most of these have been *in vitro* experiments, others have been *in vivo*. Evidence from a wide variety of tissues and cells suggests that steroid hormone receptors are transcription factors that can be activated by a wide variety of factors, only one of which is cognate hormone. Furthermore, ligand-independent activation of neural steroid hormone receptors, rather than being a pharmacological or *in vitro* curiosity, seems to be a process that occurs in the normal physiology of animals. Thinking of steroid hormone receptors only as ligand-activated proteins may constrain our thinking about the many factors that may activate the receptors and cause receptor-dependent changes in neural gene expression and neuroendocrine function. (*Endocrinology* 145: 1075–1081, 2004)

WITHIN THE DISCIPLINE of endocrinology, there is general agreement that most effects of steroid hormones on cells are mediated by, and specificity is conferred by, steroid hormone receptors at a variety of intracellular sites. As endocrinologists, we have often focused on what hormones do, that is, which hormone induces which response. Historically, much of the information about the particular functions of each hormone comes from gland extirpation and hormone replacement. A basic understanding of endocrinology tells us that removal of the gland should eliminate the constellation of physiological and behavioral responses dependent upon the secretions of that gland; replacement of the appropriate hormone should be necessary to restore the particular behavior. However, how does our thinking change if neurotransmitters, growth factors, and other factors, acting through their own membrane receptors, are able to substitute for steroid hormones and activate the steroid hormone receptors through second messenger pathways even in the absence of the steroid hormones? What if this allows the environment, acting through neurotransmitter and intracellular signaling pathways, to cause changes in behavior and physiology that had been thought of as hormone dependent?

This minireview will focus on the idea that regulation of the activation of neuronal steroid hormone receptors in the absence of hormone must be taken into account in experiments involving mechanisms of either steroid hormone action or of steroid hormone receptors in the brain (and elsewhere as well). Experiments demonstrating that particular steroid hormones need not be present in order for cells to express steroid hormone receptor-dependent responses will be discussed. Examples will be provided in which the receptors are activated in the apparent absence of ligand, including situations in real-life physiology, where these processes may come into play.

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Cellular Mechanisms of Hormone Action

Steroid hormone receptors have been extensively studied since they were first characterized in the 1960s and 1970s (1–5). Although steroid hormones act by a variety of cellular mechanisms in the brain, as they do elsewhere, it is clear that one mechanism involves binding to these intracellular steroid hormone receptors acting as transcriptional regulators. Being lipid soluble, steroid hormones diffuse into cells, and then they bind with high affinity to unoccupied receptors in cells in which they are available. Ligand binding to the receptor results in changes in protein-protein interactions, conformational change, activation, and dimerization of receptors through processes that have been well characterized. The hormone-receptor complex in association with coregulators, other proteins, and DNA induces transcriptional changes, leading to alterations in protein synthesis and consequently to changes in cellular function and ultimately physiological response (6–8).

Besides acting through steroid hormone receptors acting as transcription factors, steroid hormones also act in part via interaction with membrane receptors through a variety of intracellular signaling systems (9–15), and some of those membrane receptors are derived from the same gene that makes the transcription factor receptor (9, 16). These, and other classes of estrogen receptors, are discussed in another minireview in this issue (15). However, this minireview will focus only on steroid hormone receptors acting as direct, transcriptional regulators.

Ligand-Independent Activation

Although each of the steroid hormone receptors often has more than one name (*e.g.* estrogen/estradiol receptor; progestin/progesterone receptor), the receptors were named on the basis of their binding to, and activation by, each class of steroid hormone. In fact, the nuclear receptor signaling atlas website (http://www.nursa.org) defines progestin and es-

trogen receptors as progestin- and estrogen-activated members of the nuclear receptor superfamily of transcription factors, respectively. However, evidence that has accumulated over the past decade suggests that binding of steroid hormones is just one of a variety of ways that these steroid hormone receptors are activated. In 1991, Power *et al.* (17, 18) first reported the startling discovery that the chick ovalbumin upstream promoter receptor, progestin receptor, and estrogen receptor could each be activated *in vitro* by activation of D_1 dopamine receptors. The process by which dopamine and other compounds activate steroid hormone receptors via second messenger pathways is referred to as ligand-independent activation.

Both progestin receptors and estrogen receptors in a variety of cell types can be activated by these alternative, nonhormonal routes. Much of the work done *in vitro* and in peripheral tissues has focused on activation of estrogen receptors by growth factors, such as epidermal growth factor (19) and IGF-1 (20, 21). However, ligand-independent activation seems to be a common feature of steroid hormone receptors, as evidenced by the growing list of factors and signaling pathways known to activate one or both of these receptors in various cell types and tissues. In addition to growth factors, this includes, but is not limited to, protein and peptide hormones, such as insulin (22) and GnRH (23, 24), neurotransmitter agonists, such as D_1/D_5 dopaminergic agonists (18), and activators of particular intracellular signaling pathways, such as protein kinase C (25–27), protein kinase A (20, 28), MAPK (29, 30), phosphatidylinositol 3-kinase (31), and cyclin-dependent kinase (32) (for review, see Ref. 33). Most important for the present discussion are those reports supporting the idea that ligand-independent activation is a process that occurs *in vivo* under physiological conditions (21, 34–37).

There are likely to be a variety of molecular mechanisms by which ligand-independent activation of steroid hormone receptors occurs. For example, phosphorylation of Ser^{118} on the estrogen receptor α by the MAPK pathway leads to enhanced transcriptional activity (29, 30, 38). Likewise, phosphorylation of Ser^{236}, which regulates dimerization of the receptors, can be activated by protein kinase A (39). However, coactivators that influence transcriptional activity of the receptors may also be activated by some of these intracellular signaling pathways (40–42). Furthermore, phosphorylation of activation function-1 on estrogen receptor β may recruit the coactivator steroid receptor coactivator-1 (SRC-1) to the estrogen receptor (43). Similarly, transcriptional activity of progestin receptors can be increased by direct effects on the progestin receptor, by activation of coactivators (44, 45), and by decreasing interaction with nuclear corepressors, like nuclear receptor corepressor and silencing mediator for retinoid and thyroid hormone receptor (46).

Ligand-Independent Activation of Neuronal Progestin Receptors

In female rats, as in guinea pigs, mice, and hamsters, sexual receptivity is induced during the estrous cycle by the sequential release of estradiol followed by progesterone, both of which then act on specific brain regions (47). Similarly, optimal levels of sexual receptivity in ovariectomized rats are dependent on progesterone acting on a backdrop of estradiol priming. In studies of the cellular mechanisms of this regulation, the absolute necessity of progestin receptors for progesterone-facilitated sexual behavior in some rodent species has been shown by the use of progesterone antagonists (48–50), antisense oligonucleotides to progestin receptor mRNA (51–53), and progestin receptor gene disruption (54). Generally, when progestin receptor levels are elevated, animals respond to progesterone with the expression of sexual behavior. 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 (47, 55). Thus, there is strong evidence that intracellular progestin receptors serve an important role in mediating the behavioral effects of progesterone.

A variety of neurotransmitter agonists can substitute for progesterone in facilitation of sexual behavior. For example, the intracerebroventricular infusion of a D_1/D_5-specific dopamine receptor agonist substitutes for progesterone in facilitating sexual behavior in estradiol-primed rats (50). The process by which these dopamine agonists facilitate sexual behavior involve ligand-independent activation of progestin receptors (50). As with progesterone facilitation of sexual behavior, either progesterone antagonists (50), antisense oligonucleotides directed at the progestin receptor mRNA (53, 56), or progestin receptor gene disruption in mice (54) blocks this facilitation, supporting the idea that the facilitation of sexual behavior by dopamine involves ligand-independent activation of neuronal progestin receptors.

Neuronal progestin receptors are not activated only by progestins and dopamine. In fact, GnRH (57), prostaglandin E_2 (57), and nitric oxide (58) each facilitate sexual behavior in rats by a progestin receptor-dependent process, presumably ligand-independent activation. Furthermore, stimulation of cAMP (57) and cyclic GMP (59) also each facilitate the expression of feminine sexual behavior by a progestin receptor-dependent mechanism, implicating protein kinase A and protein kinase G pathways in ligand-independent activation of neural progestin receptors and subsequent sexual behavior. Although the involvement of these signaling pathways has been tested only by the ability of a progestin antagonist to block facilitation, the evidence is consistent with the more extensive literature on dopaminergic activation of progestin receptors.

The idea that many compounds that facilitate sexual behavior do so by influencing a common second messenger system was first proposed by Whalen and Lauber (60) with respect to cyclic GMP and by Beyer and Gonzalez-Mariscal (61) with respect to cAMP in 1986. The more recent reports carry this idea forward to implicate progestin receptors in the final common signaling pathway with which each of these, and perhaps other, signaling pathways interact (36). Furthermore, the phosphatase-1 inhibitor, dopamine and cAMP-regulated phosphoprotein (molecular mass, 32 kDa) (DARPP-32) (62), has been implicated in the process by which progestin receptors are activated, and sexual behavior is induced, by either progesterone or ligand-independent activation (63).

Ligand-independent activation of progestin receptors is not just a pharmacological or *in vitro* curiosity. It may 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 male rats, for example, for 15 min at a time followed by 15 min away from the male (64), their sexual receptivity increases over the course of a few hours, a response that is not dependent on progesterone secretion from the ovaries or the adrenal glands (65). Treatment with a progestin antagonist just before exposure to the male rats completely eliminates this response (65). This suggests that stimulation from the social environment (*i.e.* in this case, genitosensory, mating stimulation) enhances subsequent sexual behavior through ligand-independent activation of progestin receptors. Furthermore, the results suggest that, in addition to being activated pharmacologically by neurotransmitter agonists, neuronal progestin receptors can undergo ligand-independent activation *in vivo* by a physiologically relevant stimulus. Interestingly, neuronal expression of the immediate-early protein Fos in response to genitosensory stimulation is also blocked by progesterone antagonists (65, 66), suggesting that, in some neurons containing progestin receptors, the response of immediate-early genes to afferent input is gated by ligand-independent activation of progestin receptors.

Ligand-independent activation of neural progestin receptors is not limited to regulation of sexual behavior. This process is also involved in the regulation of ovulation during the rat and mouse estrous cycle (67). 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 (67, 68). In this case, the afferent stimulation, which activates the progestin receptors, is believed to be the endogenous, circadian, neuronal signal required for the preovulatory gonadotropin surge.

The author of this minireview would be negligent if steroid hormones synthesized in the brain were not discussed as an alternate hypothesis to ligand-independent activation to explain the effects discussed. It is now known that progesterone is synthesized in the nervous system, including the brain (69, 70), and like the synthesis of progestin receptors, synthesis of progesterone may even be regulated by estradiol under some circumstances (71). Although we cannot completely exclude the alternate hypothesis that the treatments that block progestin receptors also block the binding of progesterone that might be synthesized in the brain to progestin receptors, results of an immunocytochemical study argue against it. We observed that the immunocytochemical changes in the progestin receptor induced by progesterone in estradiol-primed, ovariectomized/adrenalectomized rats are not seen in response to a genitosensory stimulus that activates forebrain progestin receptors (72). Because the genitosensory stimulus did not cause the immunocytochemical changes in the progestin receptors, the results are consistent with the idea that it did not induce progesterone synthesis in the brain. Therefore, the results of this experiment support the idea that a variety of signaling pathways may induce progestin receptor-dependent changes in the brain in the absence of progesterone.

Ligand-Independent Activation of Neuronal Estrogen Receptors

Ligand-independent activation of neural steroid receptors is not limited to progestin receptors. In fact, in a wide variety of cell types and tissues, including the brain, estrogen receptors can be activated by the ligand-independent route as well (Fig. 1). A recently developed, transgenic strain of mouse with a luciferase transgene driven by a promoter containing estrogen response elements has been used to study both estradiol activation and ligand-independent activation of estrogen receptors (73). Although exogenous estradiol treatment induced the expected increase in transgene expression in a variety of tissues, the results during the estrous cycle were not as predicted. Transgene expression peaked following estradiol secretion during the proestrous stage of the estrous cycle in some tissues, such as the uterus, ovaries, hypothalamus, and liver. Surprisingly, in other tissues, especially bone and the rest of the brain, estrogen receptor-dependent transgene expression peaked well before the increase in estradiol secretion. An estrogen antagonist blocked both the estradiol-induced transgene response, as well as the response seen in the absence of estradiol, suggesting that activation of the transgene is, in fact, estrogen receptor dependent, but not estrogen dependent. These results suggest the testable hypothesis that some estrogen receptors, including some of those in the brain, are activated principally by factors other than estradiol. The data are consistent with the idea that the process of ligand-independent activation may drive the activation *in vivo* of a variety of receptors.

There are other situations where ligand-independent activation of steroid receptors could be considered in the cel-

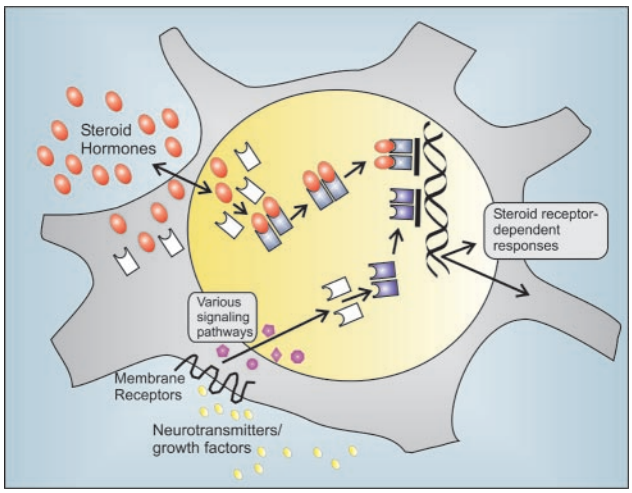

FIG. 1. Model of steroid receptor mechanism of action in neurons showing two routes of activation. In the model, only homodimers are only shown between either ligand-activated receptor or ligand-independent-activated receptors. However, it is possible that heterodimers are also formed, and that these potential heterodimers could have different transcriptional functions. In this model, for simplicity, only activation of the receptors, themselves, is shown. However, in *Ligand-Independent Activation of Neuronal Estrogen Receptors*, the ligand-independent activation of coactivators, which, in turn, influences transcriptional activity of the receptors, is discussed as one of the mechanisms by which ligand-independent activation can influence transcriptional activity of the receptors.

lular mechanisms of interactions between neurotransmitters and/or growth factors and intracellular steroid hormone receptors. An example of this is the work of Garcia-Segura and colleagues (74–79), who have elaborated the interactions between IGF-1 and estrogen receptors in the brain. It had previously been shown that estradiol regulates the concentration of receptors for IGF-1 in some brain areas (74), and that either estrogen receptor α or estrogen receptor β is coexpressed with IGF-1 in neurons and/or glia in many brain areas (75). Relevant to the present discussion, the effects of IGF-1 on neurogenesis in the dentate gyrus of adult female rats are blocked by treatment with the estrogen antagonist ICI 182,780 (76), and the effects of IGF-1 on hypothalamic neurons *in vitro* are blocked either by the estrogen antagonist or antisense oligonucleotides to estrogen receptors (77). Likewise, hypothalamic neurodegeneration induced by kainic acid is blocked by treatment with IGF-1 or estradiol. The protection against neurogeneration by IGF-1 seems to be mediated by estrogen receptors, because an estrogen antagonist blocks the effects of IGF-1 in this case too (78), suggesting that estrogen receptors mediate the response to IGF-1. Interestingly, IGF-1 treatment results in an increase in complexes of IGF-1 receptor, estrogen receptor-α, and phosphatidylinositol 3-kinase in the brain (79). Although there are alternate interpretations of these results, collectively they are consistent with the idea that some of the neuronal effects of IGF-1 are dependent upon estrogen receptors, and could be mediated at least in part by ligand-independent activation of them.

Another growth factor may influence sexual behavior via interaction with neuronal estrogen receptors. Epidermal growth factor has been reported to activate feminine sexual behavior in the absence of estradiol (80). This is a topic that has not been studied extensively, and is in need of additional experimental attempts to bypass the dependence of estradiol for activation of the neural estrogen receptors essential for the expression of feminine sexual behavior.

Regulation of Steroid Receptor Concentrations by Afferent and Efferent Influences

It has been known for quite some time that particular neurotransmitters and sources of afferent input can alter the concentration of particular steroid hormone receptors in discrete neuronal populations (47). For example, noradrenergic transmission regulates the concentrations of neural progestin (81, 82) and estrogen receptors (83), and muscarinic receptors regulate the concentration of estrogen receptors (84). Removal of the olfactory bulbs increases the concentration of estrogen receptors in the amygdala (85), and knife cuts of some afferents to the hypothalamus increase the concentration of estrogen receptors in the hypothalamus (86). In an interesting demonstration of the social environment regulating receptor levels, exposure to the odor of male prairie voles increases the concentration of estrogen and progestin receptors in the preoptic area of female prairie voles (87, 88). Likewise, exposure to mouse pups increases the concentration of estrogen receptors in some neuroanatomical regions (89, 90). Very little is known about the cellular mechanisms by which steroid hormone receptors are regulated in each of these situations.

A concept that has not been studied with respect to neural estrogen or progestin receptors, but has bearing on the discussion of factors that can regulate steroid hormone receptors, is the finding that the concentrations of androgen receptors in some spinal motoneurons are regulated by their efferent targets. For example, axotomy of, or inhibition of axonal transport in the neurons of the spinal nucleus of the bulbocavernosus, decreases the concentration of androgen receptors in the nucleus (91, 92). Reinnervation of the target muscles restores the levels, and application of brain-derived neurotrophic factor to the cut axons prevents or restores the decline (92, 93). Collectively, the data suggest the steroid hormone receptor levels in some neurons are also regulated by factors originating in the efferent targets of those neurons.

Although there have been many demonstrations of regulation of steroid hormone receptor concentrations by the environment, neurotransmitters, and other factors, there are far fewer examples of afferent input into steroid receptor-containing neurons influencing the transcriptional activity of the receptors. This is perhaps in large part due to the prevailing dogma that steroid hormone receptors are mainly ligand-activated transcription factors. Nevertheless, the number of situations discussed in which afferent input regulates neuronal steroid hormone receptors confirms the idea that influences of neurotransmitters on steroid hormone receptors are common.

Do We Constrain Our Thinking when We Tend to Think of Steroid Hormone Receptors Mediating the Effects of Steroid Hormones?

The fact that so many situations have been observed in which neurotransmitters or environmental stimuli regulate the concentrations of steroid hormone receptors, and so many intracellular signaling pathways are capable of activating steroid hormone receptors, allows us to predict that many cases will probably be found in which neurotransmitters or environmental factors activate and increase the transcriptional activity of neural steroid hormone receptors. Although these possibilities have not been investigated, there are a number of physiological situations in which ligand-independent of activation is potentially involved in mediating physiological response to afferent input from the environment or neurotransmitters. Does activation of steroid receptors underlie induced ovulation in reflex ovulators, as it seems to underlie the processing of the neuronal signal required for GnRH secretion in spontaneous ovulators discussed above (67)? Does activation of steroid hormone receptors by various neurotransmitters, perhaps dopamine (94), underlie hormone-independent induction of maternal behavior, a neuroendocrine end point often associated with estrogen receptors (95)? Is persistence of androgen-dependent copulatory behavior after castration, which is seen in many species (96, 97), referable to persistent ligand-independent activation of steroid hormone receptors by social stimuli? Will other responses in which neural estrogen and/or progestin receptors mediate the effects of estrogens or progestins be found to be regulated by neurotransmitters

and/or environmental stimuli? The possible influences of steroid receptors on sexual desire, aggressive behavior, depression, and cognitive function are but a few situations where we can look for potential influences of neurotransmitters and other factors on ligand-independent steroid receptor activation.

Another potential situation in which neurotransmitters may activate steroid hormone receptors can be seen in some old data. In 1986, we reported that injection of a dopamine-β-hydroxylase inhibitor (U-14,624) increased the concentration of both progestin receptors (98) and estrogen receptors (99, 100) tightly associated with cell nuclei. Tight association of the receptors with cell nuclei may be considered to be analogous to activation. When this work was published, there was no obvious explanation for this peculiar result, because activation without ligand did not seem reasonable. However, it is possible that the increased hypothalamic dopamine level (101) secondary to inhibition of norepinephrine synthesis had activated the steroid hormone receptors in a ligand-independent manner.

Although only a few situations have been characterized so far in which ligand-independent activation of neuronal progestin or estrogen receptors regulates behavior or physiology, two future events can be predicted. First, preliminary data suggest that other signaling pathways besides dopamine are involved. Therefore, it is likely that, as other pathways are investigated, many will be found that interact with steroid receptors by ligand-independent activation. Second, although the only estrogen receptor-dependent neuronal responses that have been directly investigated so far are feminine sexual behavior and ovulation, it is likely that many other responses that are influenced by ligand-independent activation of steroid hormone receptors will be found, when additional end points of steroid receptor activation in the brain are studied. When we begin to think about the steroid receptors as transcriptional regulators that are regulated only in some cases by steroid hormone ligand, we will likely discover many neurotransmitters and environmental stimuli that activate them.

Steroid receptors can be activated by circulating hormones, so why might receptors in some neurons also be activated by afferent input from neurotransmitters and other factors? This question can be answered on two levels—from the adaptive perspective and from the evolutionary perspective. The adaptive cause may be 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 particular steroid receptor-dependent neuronal and behavioral responses. This is best seen in the example of mating stimulation from males enhancing sexual receptivity, as was discussed earlier. The process may occur in the absence of hormones, and it may also occur in the presence of hormones.

The question can also be answered from an evolutionary perspective. Escriva *et al.* (102) argue that nuclear (transcription factor) receptors gained their ligand-binding ability during evolution. Thus, it should come as no surprise that the receptors are activated by multiple routes, only one of which is binding of cognate ligand. Interestingly, the first vertebrate steroid receptor was an estrogen receptor, and the second was a progestin receptor (103).

Conclusions

We have been too limited in our view that neural estrogen receptors and progestin receptors, acting as transcription factors, are activated solely by binding to cognate ligand to influence sexual behavior and other brain functions, despite a wealth of evidence from a variety of sources that they can be activated by many other routes. At any given time, activation of the complement of a particular steroid hormone receptor in a particular cell may be a composite of all of the factors that are impinging on that cell that induce either ligand-dependent and ligand-independent activation. Every neuron potentially has a more-or-less unique set of factors influencing it at a given moment. As an illustration, some steroid receptor-containing neurons may be receiving active dopaminergic stimulation at a particular time, but most are not. Some neurons with appropriate membrane growth factor receptors may transduce a growth factor stimulus, whereas others do not. Some neurons may have receptors activated by both ligand-dependent and ligand-independent pathways at a given time. The total activation of receptors may be a composite of activation by ligand-dependent and ligand-independent routes (104). Stated another way, through the process of activation, the steroid hormone receptors may integrate the sum of hormonal, humoral, and environmental factors. When we factor in the complexity of the regulation of activation of steroid hormone receptors by their coregulators, as well as the coupling and uncoupling of neurotransmitter receptors to intracellular signaling pathways, it is clear that each cell is capable of exquisite fine-tuning of steroid hormone receptor-dependent gene regulation.

To summarize, it is clear that steroid hormone receptors can be activated by a variety of signaling pathways. Although these relationships have been demonstrated primarily *in vitro*, it is likely that more cases of hormone-independent activation of neural steroid hormone receptors *in vivo* will be discovered. These nonhormonal influences on the receptors may act in place of, or in addition to, hormone-dependent activation of the receptors. This all leads us to conclude that steroid hormone receptors aren't just for steroid hormone anymore. They never were; our naiveté made us think that they were.

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