Mechanisms of Progesterone-Induced Neuroprotection

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ABSTRACT: Gonadal steroid hormones such as estrogen and progesterone can no longer be considered strictly within the confines of reproductive function, and with respect to their anatomic targets, extrahypothalamic structures within the brain such as the cerebral cortex have revealed themselves to be important targets. As such, it may come as no surprise that the decline in such hormones, which occurs after the menopause or ovariectomy, can result in neuronal dysfunction. Although estrogen has been shown to help restore the deficits consequent to ovariectomy, it is important to consider that ovariectomy, like the menopause, results in the precipitous loss of not only estrogen but of progesterone as well. As such, the loss of progesterone may contribute to the deficits observed after ovariectomy or the increased risk for Alzheimer's disease seen after the menopause. Indeed, recent evidence supports the neuroprotective potential of progesterone itself. Here, we review the current understanding of some of the diverse mechanisms by which progesterone may reduce neuronal vulnerability to toxic insults relevant to age and age-associated diseases such as Alzheimer's disease. Further, we comment on the need to carefully consider the various preparations of progestins that are currently available and argue that "not all progestins are created equal," at least when it comes to influences on neuroprotection and other extrahypothalamic brain functions.

KEYWORDS: progestins; progesterone; neuroprotection; signal transduction

Progesterone is a major gonadal hormone that is synthesized primarily by the ovary (corpus luteum) in the female and by the testes and adrenal cortex in the male. Although progesterone levels are generally higher in the female, it is worth noting that levels of progesterone during the female follicular phase of the menstrual cycle are similar to those seen in males, suggesting

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that progesterone plays an equally important role in both males and females. The paradigmatic mechanism by which progesterone elicits its effects is through the progesterone receptor (PR), which like estrogen receptors (ERs), has classically been described as a nuclear transcription factor, acting through specific progesterone response elements (PREs) within the promoter region of target genes to regulate transcription. Such a mechanism may be relevant to the regulation of neurotrophin expression, which also appears to be regulated by progesterone.[1]

Two major isoforms of the classical progesterone receptor exist, PR-B, and its N-terminally truncated form, PR-A. The latter has been shown to exert negative control of not only PR-B-mediated transcription but that mediated by the ER and glucocorticoid receptor as well. This negative regulation of ER function by a PR may underlie, at least in part, the mechanism by which progestins functionally antagonize the effects of estrogen. For example, progestins inhibit estrogen's ability to increase serum levels of 1,25-dihydroxy vitamin D,[2] whose consequence may be to antagonize estrogen's beneficial effects on bone. However, the interaction between the two receptors may not only result in transrepression but may also be cooperative in nature. For example, Migliaccio *et al.*[3] demonstrated a physical interaction of the progesterone receptor with the ER in mammary tumor cells and that this association was necessary for progesterone to elicit the activation of the mitogen-activated protein kinase (MAPK) pathway. Further, the ability of progesterone to stimulate the MAPK pathway was blocked not only by a PR antagonist but also by an ER antagonist.[3]

As introduced in the preceding paragraph, progesterone can also elicit its effects via nongenomic mechanisms (such as the activation of typically growth factor–associated signal transduction pathways). The growing list of second-messenger/signal transduction systems activated by progesterone includes cAMP/PKA,[4] MAPK (ERK1/2),[3,5] and the PI-3K/Akt pathway.[5] Activation of such signaling pathways may not only be relevant to how progesterone regulates cellular function related to reproduction but may also be an important mechanism by which progesterone elicits its neuroprotective effects. For example, progesterone-induced neuroprotection has not only been correlated with activation of the MAPK and Akt signaling pathways[6,7] but has also been shown to depend on the activation of the MAPK pathway.[1]

As mediators of these nongenomic effects, the classical receptor has been implicated, but depending on the cellular context, a novel receptor system for progesterone may also be involved. For example, progesterone may exert its effects via interactions with membrane binding sites, characterized in the brain by the demonstration of specific, displaceable binding in synaptosomal membrane preparations.[8,9] Such membrane binding sites may include the recently cloned membrane progesterone receptor that exhibits characteristics of G protein–coupled receptors.[10,11] Progesterone, through its metabolites, can also interact with membrane-associated receptors coupled to ion channels,

such as the $GABA_A$ receptor system (see Ref. 12 for review). Such metabolites include allopreganolone (or 3α,5α-tetrahydroprogesterone), which can bind to discrete sites within the hydrophobic domain of the $GABA_A$ receptor complex and result in the potentiation of GABA-induced chloride conductance—and in turn may regulate cellular excitability and thus, excitotoxicity. Thus, progesterone's ability to interact with specific sites within the membrane (either membrane-binding sites [receptors] or with the $GABA_A$ receptor), as well as with specific cytosolic signal transducers, may help explain some of the rapid effects of progesterone, which in addition to its classical genomic mechanisms, may be important for regulating cell viability. Alternatively, the parent compound, progesterone, may also have effects on the $GABA_A$ receptor, albeit indirect. For example, progesterone may influence the $GABA_A$ receptor via the activation of a signal transduction pathway, which in turn, influences GABA-gated currents through phosphorylation of discrete sites within certain subunits of the $GABA_A$ receptor.[13,14]

PROGESTERONE-INDUCED NEUROPROTECTION

A considerable amount of information has been obtained regarding the mechanisms underlying estrogen's protective effects. One experimental model that has been valuable in the validation of the hypothesis that estrogens are beneficial is the use of the ovariectomized animal. Ovariectomy results in impaired cellular function that is reflected by behavioral, neurochemical, and molecular deficits consistent with those seen with advanced age or in certain age-associated diseases like Alzheimer's disease. Estrogen treatment of ovariectomized animals at least partially normalizes the deficits.[15–17] It is important to recognize, however, that ovariectomy results in the loss of not only the primary forms of circulating estrogen but also of another major ovarian hormone, progesterone. Thus, the behavioral, neurochemical, and molecular deficits that resulted from ovariectomy may not only have been due to a loss in circulating estrogen levels but may also have been a consequence of progesterone loss. Moreover, estrogen replacement does not always lead to the complete recovery of the ovariectomy-induced deficit.[17] As such, this partial normalization could be a result of not having replaced the other steroid hormones similarly lost following ovariectomy.

In humans, the menopause is also characterized by the concomitant loss of progesterone, and not just estrogen. As such, the increased risk for developing Alzheimer's disease may be contributed by the precipitous decline in both estrogen and progesterone levels. Thus, it is possible that progesterone is equally beneficial, either alone or in conjunction with estrogen.

In fact, progesterone, like estrogen, has been reported to have neuroprotective effects in various experimental models. In hippocampal neurons, both es-

tradiol and progesterone were shown to reduce neuronal vulnerability to such insults as glutamate, $FeSO_4$, and Aβ toxicity.[18] In addition, secondary neuronal loss following cortical contusion injury and resulting cognitive impairment was significantly reduced in mice that received progesterone treatment relative to that of untreated controls.[19,20] Progesterone was also effective at reducing the amount of cell death seen in an acute model of global ischemia.[21] Further, progesterone was protective against excitotoxic insult and promoted morphological and functional recovery in the Wobbler mouse, an animal model of spinal cord degeneration.[22,23]

Mechanistically, progesterone-induced neuroprotection may be mediated by multiple mechanisms. The effects of progesterone on neurotrophin expression may be mediated by the classical mechanism of transcriptional activation. Alternatively, progesterone may act through novel receptor systems (membrane PR or the $GABA_A$ receptor) to regulate cellular events that are important for neuroprotection. For example, metabolites of progesterone, such as allopregnanolone, can bind to a site within the $GABA_A$ receptor complex, and as a consequence, potentiate the effect of GABA on its receptor (see Ref. 12 for review). This activation of the $GABA_A$ receptor, in turn, has been shown to modulate cell survival, particularly in models of excitotoxicity, and may be consistent with the protective effect of progesterone seen against kainate-induced seizure activity and subsequent cell death.[24] Progesterone may also be protective through its ability to elicit the activation of specific signaling pathways relevant to neuroprotection,[5,6] as well as increasing the expression of antiapoptotic proteins such as Bcl-2.[6] Finally, progesterone has been described to have antioxidant effects[25] that may also contribute to neuronal survival following injury. Collectively, these data support the multiple mechanisms by which progesterone is protective and supports the importance of progesterone, either alone or in combination with estrogen, in promoting cell survival.

CLINICAL USES OF PROGESTINS

The major form of progestin used in hormone therapy (HT) is the synthetic compound medroxyprogesterone acetate (MPA), which is the major progestin used in the formulation of hormone therapy and oral contraceptives. With regards to HT, the role of the progestin is to counteract the uterotrophic effects of estrogen or an apparent increase in risk for certain cancers like uterine cancer resulting from unopposed estrogen treatment (for review, see Ref. 26). The natural hormone, progesterone (Prometrium®), is also used, though to a lesser degree in the United States. Although both the synthetic progestins and the natural hormone, progesterone, can elicit similar effects (i.e., both can inhibit the uterotrophic effects of estrogen and can exert an inhibitory in-

fluence [negative feedback] on gonadotropin secretion at the level of the hypothalamus), it is important to recognize that these hormones do exhibit important differences, particularly in relation to their effects on the brain. For example, progesterone has been described to be neuroprotective,[6,18] whereas the synthetic progestin, MPA, was not.[6] Moreover, MPA antagonized the effects of estrogen, whereas the natural hormone progesterone did not.[6,7,27] Such differences may be important in considering the results of the recently published WHI studies which used MPA rather than progesterone, and further, could provide critical insight into the development of the most effective therapeutic formulations for the treatment of various postmenopausal conditions.

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