Endocrine, *vol. 29, no. 2, 271–274, April 2006* 0969–711X/06/29:271–274/$30.00
ENDO (Online) ISSN 1559-0100

Progesterone-Induced Neuroprotection

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Estrogen and progesterone are two steroid hormones whose biology has been greatly studied within the confines of reproductive function. As a consequence, the effects of these hormones on the brain have focused primarily on the hypothalamus. Growing evidence, however, forces us to recognize that various extrahypothalamic brain regions, including the cerebral cortex and hippocampus, are equally important targets of these hormones. As such, hormones are involved in numerous aspects of brain function, and elicit effects ranging from the regulation of mood and cognition to the regulation of neuronal survival. While estrogen exerts neuroprotective effects in various experimental models, the potential for progesterone as a protective agent has, until recently, been greatly understudied. Here, we review the data from various laboratories including our own that support the protective role of progesterone and describe the multiplicity of mechanisms by which progesterone elicits these protective effects. Finally, we contrast the neurobiology of progesterone with that of the clinically used progestin, medroxyprogesterone acetate (MPA), and suggest that the "natural" progesterone may be the better choice when considering which progestin to use for future therapeutic/palliative purposes in CNS-related disorders.

Key Words: Progestins; progesterone; neuroprotection; signal transduction.

The Biology of Progesterone

Progesterone is a major gonadal hormone present in the general circulation of both males and females. The classical mechanism by which progesterone elicits its effects is via the progesterone receptor (PR), which like estrogen receptors (ER), has been described as a nuclear transcription factor. The "classical" PR is found in both cytosolic and nuclear compartments of a cell, and, as a nuclear transcription factor, acts through specific progesterone response elements (PRE) within the promoter region of target genes to regulate transcription of such genes. Two isoforms of the PR are known: PR-B and its N-terminally truncated form PR-A. The latter can antagonize not only PR-B–mediated transcriptional activation, but that mediated by the ER as well, and thus, may underlie at least in part, the mechanism by which progestins functionally antagonize the effects of estrogen. However, the nature of interaction between the receptor systems may not always be antagonistic, but can also be cooperative. For example, Migliaccio et al. *(1)* demonstrated a physical interaction of the PR 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. Furthermore, the ability of progesterone to stimulate the MAPK pathway was not only blocked by a PR antagonist, but also by an ER antagonist *(1)*.

While the classical PR clearly plays an important role in mediating the effects of progesterone, evidence also exists for alternative mechanisms of action, including that which involves a putative membrane progesterone receptor. Because progesterone has a log*P* value (the octanol/water partition coefficient, an index of lipid solubility) of about 4, the interpretation is that for every 10,000 molecules of progesterone, only one partitions into the aqueous environment (and therefore, the cytosol). Therefore, the dogma which states that progesterone traverses the plasma membrane's lipid bilayer to interact with cytosolic receptors *because* it is lipophilic may not be completely correct. Instead, the physicochemical properties of progesterone may support the involvement of a membrane PR in mediating its effects. In fact, specific, displaceable binding sites for steroid hormones have been observed in synaptosomal membrane preparations *(2,3)*. However, only recently has a *bona fide* membrane progesterone receptor been identified and cloned *(4)*. Not only did Zhu and colleagues discover a novel membrane-associated progesterone receptor, but this receptor was predicted to contain a seven-transmembrane-spanning domain, and is coupled to the Gi/o class of G proteins *(5)*. Other proposed membrane progesterone receptors include 25-Dx, an apparently neuron-specific membrane progesterone receptor *(6–8)* that is involved in numerous aspects of cell function ranging from neuronal development *(9)*, steroidogenesis *(10)*, and regulation of reproductive behavior *(6)*.

Received October 24, 2005; Accepted October 24, 2005.

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Progesterone can also elicit its effects through its metabolites. For example, the 5α-reduced metabolite of progesterone, allopregnanolone, binds to discrete sites within the hydrophobic domain of the $GABA_A$ receptor complex, and results in the potentiation of GABA-induced chloride conductance (see ref. *11* for review). Additionally, progesterone can promote the recruitment of second messenger/signal transduction systems, including cAMP/PKA *(12)*, MAPK *(1,13)*, and PI-3K/Akt *(13)* pathways. All these mechanisms of action may be relevant to the ability of progesterone to influence the vulnerability of brain cells to neurotoxic insult, and is discussed in greater detail below.

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 validating the hypothesis that estrogens are beneficial, is 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 *(14–16)*. It is important to recognize, however, that ovariectomy results in not only the loss of the primary forms of circulating estrogen, but also results in the loss 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 of circulating estrogen, but may also have been a consequence of a decline in progesterone levels. Moreover, estrogen-replacement does not always lead to the complete recovery of the ovariectomy-induced deficit *(16)*. 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 ovarian estrogen secretion, as well as ovarian luteal phase progesterone. As such, the increased risk for developing Alzheimer's disease following the menopause may be contributed by the decline in *both* estrogen and progesterone levels. Thus, it is possible that progesterone may be equally beneficial, either alone or in conjunction with estrogen.

In fact, progesterone has been reported to have neuroprotective effects in various experimental models. In hippocampal neurons, for example, progesterone reduced neuronal vulnerability to such insults as glutamate, $FeSO_4$, and Aβ toxicity *(17)*. In addition, secondary neuronal loss following cortical contusion injury and resulting cognitive impairment was significantly reduced in mice that received progesterone treatment relative to untreated controls *(18,19)*. Progesterone is also effective at reducing the amount of cell death following an acute episode of global ischemia *(20)*, and may be related to the ability of progesterone to reduce lipid peroxidation, the generation of isoprostanes *(21)* and the expression of proinflammatory genes *(22)*.

Progesterone also has the potential to induce remyelination as evidenced by the increased expression of myelin proteins in the damaged sciatic nerves of young adult rats and in 22–24-mo-old males with nerve crush injuries treated with progesterone *(23)*, and thus may be of therapeutic benefit for various demyelinating diseases. Furthermore, progesterone protects against excitotoxic insult and has been shown to promote morphological and functional recovery in the Wobbler mouse, an animal model of spinal cord degeneration *(24,25)*. Collectively, all these mechanisms may be important in protecting the brain against various insults relevant to age- and neurodegenerative disease–related brain dysfunction.

The protective effects of progesterone may be mediated by multiple mechanisms. The classical genomic mechanism of progesterone action, for example, may be involved in the regulation of neurotrophin expression *(26)*, which, in turn, would promote cell survival. Alternatively, progesterone may act through novel receptor systems (membrane PR or the $GABA_A$ receptor) to regulate other cellular events that are equally important for neuroprotection. Progesterone, through its metabolites, can interact with membrane-associated receptors coupled to ion channels, such as the $GABA_A$ receptor system (see ref. *11* for review). Such metabolites include allopregnanolone (or 3α, 5α-tetrahydroprogesterone), which bind to discrete sites within the hydrophobic domain of the $GABA_A$ receptor complex, and result in the potentiation of GABA-induced chloride conductance. Alternatively, the parent compound, progesterone, may also have nonallosteric influences on the $GABA_A$ receptor. 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 *(30, 31)*. Because the regulation of the $GABA_A$ receptor has been shown to modulate cell survival, particularly in models of excitotoxicity, the regulation of the $GABA_A$ receptor by progesterone may be relevant to the protective effect of progesterone seen against kainate-induced seizure activity and subsequent cell death *(27)*.

Progesterone may also be protective through its ability to elicit the activation of specific signaling pathways relevant to neuroprotection *(13,28)*. The growing list of second messenger/signal transduction systems activated by progesterone include cAMP/PKA *(12)*, MAPK (ERK1/2) *(1, 13)*, and the PI-3K/Akt pathway *(13)*, all of which have been implicated in mediating neuroprotective effects. Progesterone-induced neuroprotection has not only been correlated with activation of the MAPK and Akt signaling pathways *(28,29)* but has also been shown to depend on the activation of the MAPK pathway *(26)*. Activation of these signaling

pathways may also lead to increased expression of antiapoptotic proteins such as Bcl-2 *(28)*.

As mediators of these nongenomic effects, the classical receptor has been implicated, but depending on the cellular context, a novel receptor system for progesterone could also be involved. 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 *(2,3)*. Such membrane binding sites may include the recently cloned membrane progesterone receptor that exhibits characteristics of G protein–coupled receptors *(4,5)*. 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.

Finally, progesterone has been described to have antioxidant effects *(21)* that could also afford protection of brain cells against age-related neurodegenerative disorders and various forms of injury. Collectively, these data describe multiple mechanisms that are elicited by progesterone, and strongly support the ability of progesterone, either alone, or in combination with estrogen, to promote cell survival.

Medroxyprogesterone Acetate and Progesterone

Medroxyprogesterone acetate (MPA) is a synthetic progestin often used in conjunction with estrogens to reduce the risk of certain cancers (uterine cancer, for example) resulting from unopposed estrogen therapy *(32,33)*. However, it is important to recognize that the biology of progestins in extraneural tissue may not be identical to that seen in brain tissue. Consistent with this idea is the fact that MPA is not cytoprotective, whereas progesterone, as outlined above, has potent protective effects. Progesterone, but not MPA, protects against glutamate toxicity in primary dissociated hippocampal neurons *(28)*. Accordingly, progesterone, but not MPA, prevents the increase in intracellular Ca^{2+} levels consequent to glutamate exposure. Although both progesterone and MPA elicit an increase in ERK phosphorylation, only progesterone elicited the nuclear translocation of ERK, suggesting that nuclear translocation of the activated kinase is necessary for progesterone's protective effects *(29)*. Furthermore, not only does MPA fail to mimic the ability of progesterone to increase the expression of the antiapoptotic protein, Bcl-2, but actually inhibits ability of estrogen to do so *(28)*.

This disparity between the effects of P4 and MPA has also been observed in vivo. A study using rhesus monkeys illustrated that the combined administration of estrogen and progesterone protects against coronary vasospasm, whereas the co-administration of MPA with estrogen obviated this effect *(34)*. This inconsistency with respect to the effects of progesterone and MPA is also evident in humans, where progesterone administration to postmenopausal women enhances the protective effects of estrogen on exercise-induced myocardial ischemia whereas MPA does not *(35)*. All together, these studies highlight the fact that the neurobiology of progesterone is different from that of MPA. Although both progesterone and MPA may be effective at reducing the uterotrophic effects of unopposed estrogen, MPA does not have similar effects as progesterone in the brain. 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.

Conclusion

With age, circulating gonadal hormone levels decline in both males and females. In women, however, such age-associated decreases are much more dramatic as a result of the menopause. The menopause occurs at an average age of 54, and is characterized by a precipitous decline in circulating gonadal hormones. Because the average lifespan of women has increased to approx 80 yr of age (well past the average age at which the menopause occurs), a substantial portion of a woman's life is spent in a hormone-deprived state. Given that many neuronal populations are normal targets of hormones such as progesterone, it is not surprising that a loss of such hormones may render an organ system such as the brain more vulnerable to insult, or an individual more susceptible to neurodegenerative diseases like Alzheimer's disease. The information presented here describes that progesterone is indeed protective and that such protection can be afforded through multiple mechanisms. While the biology of progestins in reproductive tissue has been well studied (due to the historical emphasis that hormones regulate reproductive function), the mechanisms in play in CNS tissue may not be identical. The data presented here clearly support the potential application of progesterone for preventing neuronal dysfunction. Additional studies that expand our understanding of how progestins influence the brain will be instrumental in the development of better and safer treatments for menopausal symptoms and those brain disorders whose incidence is greater in the postmenopausal period.

Acknowledgments

Supported by NIH grants AG 22550 and AG 23330 and a Young Investigator award from NARSAD.

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