A Review of Current Research on the Effects of PROGESTERONE

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A 1997 review of a documented annotated research article[1] concludes that much is known about the effects of progesterone on the uterus but that the effects of progesterone on bone, breast tissue, and the brain are poorly understood. In this article, we review the effects of progesterone on bone, breast tissue, the brain, and the circulatory system in humans and in animal models.

Effects of Progesterone on Bone

Current studies[2] examining the role of progesterone on bone homeostasis indicate that the effects of progesterone are mediated by its nuclear receptors. In the 1970s, O'Malley et al[2] showed that those receptors are composed of two receptor proteins, PRa and PRb, each of which binds progesterone.[2] Research by MacNamara and Loughrey[3] in 1998 indicated that PRa and PRb messenger ribonucleic acid (mRNA) transcripts are expressed in human osteoblasts and that progesterone receptor promoter activity is estrogen responsive. This provides evidence that bone-forming cells are physiologically influenced by progesterone. A study[4] by Luo and Liao supports the role of progesterone in regulating the function of metalloproteinase (specifically matrix metalloproteinase [MMP]) in osteoblast cells involved in bone remodeling and resorption. MMP initiates bone resorption by degrading the bone matrix. This requires the activation of MMP-2 via a complex consisting of a membrane-type matrix metalloproteinase-1 (MT1-MMP) and a tissue inhibitor of metalloproteinase (TIMP-2) on the cell surface. In that study, progesterone was shown to increase the levels of MT1-MMP and mRNA in osteoblasts. Progesterone acted only on the MT1-MMP protein; that action may contribute to bone formation.

Progesterone binding to glucocorticoid receptors is another pathway that may modulate bone remodeling. Glucocorticoids cause bone loss by blocking osteocalcin synthesis and preventing the attachment of osteoblasts to matrix proteins such as osteonectin. Some studies[5,6] indicate that progesterone exerts an antiglucocorticoid effect by acting as a ligand for glucocorticoid receptors. A study[7,8] of progesterone replacement in very premature infants yielded exciting results with respect to the role of progesterone in bone development. During pregnancy, the plasma concentrations of estradiol and progesterone in the fetus increase by a factor of 100. A prematurely delivered infant is deprived of those hormones at an early developmental stage. Seventy years ago, estradiol supplementation was used on premature infants to promote body-weight gain, but the success of that treatment was minimal and the practice was abandoned.[7,8] However, because of the positive benefits of postmenopausal hormone replacement, the effect of estradiol supplementation on the prevention of the osteopenia of prematurity was again examined. When intrauterine levels of estradiol and progesterone were supplemented for 6 weeks postnatally in premature infants, an improvement in bone mineral accretion was demonstrated.[7,8]

Effects of Progesterone on Breast Tissue

The controversial question about whether a combination of progestins and estrogen leads to an increase in breast cancer remains unanswered. In this article, a review of the effects produced by different types of progestins is presented.

Breast tissue is of several types.[9] Epithelial tissue divides during the progesterone-dominant phase of the menstrual cycle. Ductile tissue grows and branches during pregnancy as a result of estrogen. The lobule-alveolar systems of the breast also develop during pregnancy as a result of the effect of progesterone, which causes the growth of lobules, the budding of alveoli, and an increase in the secretory capacity of alveolar cells. That process, which is termed "differentiation," is the reason for which full-term pregnancy early in life provides protection against carcinogen-induced breast cancer.[10] Cancers often develop in epithelial cells. All cells have a finite life span, and there is a balance between cell division and cell death. When stimulated by estrogen, the BCL2 gene causes breast cells to grow rapidly and prevents cell death. In ovarian carcinoma cell lines and in breast epithelial cells, progesterone induces apoptosis and upregulates the P53 gene.[11,12] Formby and Wiley[13,14] demonstrated that "progesterone at a concentration similar to that seen during the third trimester of pregnancy exhibited a strong antiproliferative effect on at least two breast cancer cell lines. Apoptosis was induced in the progesterone receptor expressing T47-D breast cancer cells."

To promote the understanding of the action of progesterone, researchers have been trying to culture human breast epithelial cells that are nontumorigenic and that contain progesterone receptors. Those receptors are synthesized in response to estrogen. Synthetic progestins such as medroxyprogesterone acetate or norethindrone occupy the progesterone receptor site and inhibit the binding of endogenous progesterone to the receptor. Synthetic progestins do not produce the P53 gene and thus would prevent the production of progesterone and the activation of the P53

gene. This chemically induced progesterone deficiency, like natural progesterone deficiency, may increase the risk of breast cancer because the BCL2 gene is upregulated by estradiol and no corresponding downregulation opposes that action. Breast epithelial cell proliferation is greater when a combination of estrogen and medroxyprogesterone acetate is used than when estrogen only or no hormone replacement therapy is used.[15] Another study[16] compared postmenopausal women who followed one of four protocols: topically applied estradiol, progesterone, estradiol plus progesterone, or placebo. The use of estradiol alone increased the proliferation index of breast epithelium 100-fold, the use of progesterone alone increased the proliferation index 15-fold, and combination treatment with estradiol plus progesterone increased that index 13-fold.

Effects of Progesterone on the Brain

We also reviewed the current literature on the effects of progesterone on the brain in adult humans and preterm infants. As noted previously, premature infants are deprived of the full benefits of estradiol and progesterone. In one study,[17] 15 preterm infants received progesterone and estradiol replacement at intrauterine levels. When the treated infants were examined, they exhibited a normal psychomotor pattern, but preterm infants who were not treated with progesterone and estradiol replacement (the control group) exhibited delayed psychomotor development. That area of research is new, and more extensive studies are needed to evaluate the potential benefits of and adverse side effects induced by the postnatal replacement of those hormones at intrauterine levels in premature infants.[17]

Research was conducted by Wagner et al[18] to determine the differences in progesterone-receptor formation in the developing male and female human brain. The brain of the human male differs structurally and neurobiologically from that of the human female. Research studies indicate that maternal progesterone (not fetal steroids such as androgens and estrogens) induces gender differences in the human brain. Maternal progesterone enters the blood and brain of the fetus, and the activation of the progestin receptor modifies the function of the brain cells. Male fetuses exhibit a greater sensitivity to progesterone and have more progestin receptors than do female fetuses. The discovery of this role of progesterone superseded the view that only androgens and estrogens are the agents of sexual differentiation. Research on neuronal differentiation, cell migration, cell death, and other cellular events that contribute to gender-based differences in the central nervous system is in progress.[18]

According to Baulieu and Schumacher,[19] progesterone is synthesized by Schwann cells and enhances myelin formation in the peripheral nerves. Those authors also confirmed that progesterone promotes myelin repair in the brain. When progesterone was given to animals with transplanted oligodendrocytes, significantly more axons were remyelinated after 3 to 5 weeks. Baulieu and Schumacher found that progesterone rapidly increased the expression of a transcription factor known as Krox-20, which plays a critical role in the regulation of the myelin gene in Schwann cells. If the *in vitro* work can be duplicated *in vivo*, it may lead to the development of specific steroid compounds used to treat male and female multiple sclerosis patients without adversely affecting other areas of the body. Significant research today is devoted to the development of a synthetic progestin for postpartum administration to women with multiple sclerosis to prevent relapses of that disease. The effect of progesterone on excitotoxic cell death, lipid peroxidation, and the induction of specific enzymes in the neurologic recovery from brain and spinal cord injury is the focus of that research. Researchers now have evidence that it may be easier for the female brain to repair itself after injury.[20] Progesterone reduces the cerebral swelling and consequent ischemia-induced cell damage that follows brain injury. According to Wright et al,[21] progesterone given by injection may be an inexpensive and safe way to protect the brain from edematous injury.

Two other key elements linked to neuroprotection lie within the signal transduction pathway in the brain. Progesterone elicits the phosphorylation of Akt, a downstream effector of the phosphoinositide-3 (PI-3) kinase pathway and of extracellular-signal regulated kinase (ERK), a component of the mitogen-activated protein kinase (MAPK) pathway. These pathways offer mechanisms for neuroprotection against various insults and may lead to discoveries for the treatment of neurodegenerative disorders such as Alzheimer's disease.[22]

Progesterone and 19-norprogesterone also protect against glutamate toxicity, in stark contrast to the lack of such protection provided by medroxyprogesterone acetate. According to Nilsen and Brinton,[23] 17β-estradiol upregulates BCL2, which prevents cell death. Progesterone and 19-norprogesterone, alone or in combination with estrogen, increase the expression of BCL2. Those authors state that "These results may have important implications for the effective use of hormone replacement therapy in the maintenance of neuronal function during menopause and aging and for protection against neurodegenerative diseases such as Alzheimer's disease.[23]

Progesterone affects the expression of several proteins in the brain. For example, progesterone stimulates γ-aminobutyric acid (GABA) signaling pathways in specific areas of the brain. In animal studies, those pathways regulate the signals in the brain that involve sexual response, but the effect of progesterone in the human brain is limited. In animal models, the most well-defined aspect of progesterone action is progesterone-receptor (PR) mediated effects in the hypothalamus and preoptic area. Those effects may also be mediated by the direct interaction of 5-α-reduced progesterone metabolites and $GABA_A$ receptors.[24]

A study[25] of the effect of finasteride, a 5-α-reductase inhibitor, in the murine model revealed that the level of allopregnenolone is very important in inhibiting the anticonvulsant and sedative effects of alcohol. Increased stress levels trigger the release of corticosterone and progesterone, from which allopregnenolone is produced. A dramatic rise in the allopregnenolone level of animal subjects was observed after they had ingested ethanol. When finasteride was administered, the formation of allopregnenolone from progesterone after ethanol ingestion was blocked. This may be the reason for which

women, who have a higher level of the steroid than do men and thus produce more allopregnenolone, can become more relaxed after consuming less ethanol than that required to relax a male counterpart. It may also explain the difference in the reactions of men and women to the ingestion of ethanol. In the study[25] cited, female rats consumed more ethanol during the phase of their reproductive cycle when their progesterone level was low.

Progesterone also affects the pulsatile gonadotropin-releasing hormone (GnRH) stimulus from the hypothalamus. GnRH pulses regulate the secretion of pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Rapid pulses of GnRH produce an LH surge that is followed by ovulation, after which the GnRH pulse slows to produce more FSH. In women with a hypothalamic dysfunction such as hypothalamic amenorrhea or polycystic ovarian syndrome, GnRH pulses can be influenced by progesterone, which slows the GnRH pulse secretion to produce an increase in the level of FSH and subsequent follicular maturation.[26]

Effects of Progesterone on the Heart and Circulatory System

Female sex hormones may confer some cardioprotective effects, because clinical observation indicates that coronary artery disease is more common in men and postmenopausal women than in premenopausal women. Estrogen deficiency is currently thought to increase the risk of coronary heart disease because many epidemiologic studies have reported a reduced risk of coronary heart disease in premenopausal women. Estrogens produce favorable changes in endothelial function, vascular reactivity, lipid levels, and blood flow. Vascular effects of added progestins ranging from neutral to detrimental have been described, but the effects of progesterone on endothelial function in humans per se have not been reported. The American Heart and Estrogen/Progestin Replacement Study (HERS) is a large multicenter randomized study of the effects of hormone replacement therapy in postmenopausal women with heart disease.[27] Conjugated equine estrogens and medroxyprogesterone acetate were used as hormone replacement therapy in that study. HERS results revealed no reduced risk of coronary heart disease in the subjects who received hormone replacement therapy but instead indicated a higher risk of events related to coronary heart disease during the first year of treatment.

When the benefits and risks of hormone replacement therapy are assessed, it is important to determine which chemicals were used as treatment because individual progestins have varying pharmacologic properties and do not produce the same side effects. Progesterone and 19-norprogesterone do not exert an androgenic action and have no negative effect on lipid levels and vascular reactivity in ani-

mal models or on exercise-induced myocardial ischemia in humans.[28-29] Estrogens preserve the normal endothelium-mediated dilation of coronary arteries, and progesterone does not reverse the effect of that potentially cardioprotective mechanism. In one animal study,[30] progesterone caused coronary relaxation by inhibiting Ca^{++} mobilization into coronary smooth muscle and by other mechanisms.

In a study by Mather et al,[31] the effect of 17-β-E_2, progesterone, and 17-β- E_2 with progesterone on endothelial forearm responses was examined. The authors concluded that progesterone does not produce detrimental vascular effects in healthy menopausal women who do not have risk factors for cardiovascular disease.

Progesterone also prevents the multiplication and migration of smooth muscle cells, which are involved in the formation of plaque that blocks arteries in the heart and brain. Progesterone inhibits deoxyribonucleic acid synthesis and the proliferation of the smooth muscle cells.[32] This finding was further supported by animal studies[33] in which ovariectomized female progesterone receptor (PRKO) knockout mice and wild-type (WT) litter mates were used to study the effects of progesterone on vascular smooth muscle cells. Progesterone produced no significant effect in the PRKO mice studied, but it inhibited the proliferation of vascular smooth muscle cells in the WT mice. That research may lead to the identification of the mechanism of action by which progesterone protects against atherosclerosis. In 1992, researchers also discovered that progesterone reduces platelet aggregation via the enhancement of nitric oxide, which is an endothelium-derived relaxing factor.[34]

In one study,[34] a direct effect on platelet aggregation was noted when rabbit aortic strips were exposed to progesterone, which promotes endothelium-nitric oxide relaxing factor. The effect of progesterone on the rat aorta and mesenteric arteries differed; this suggests that the effect of progesterone on the relaxant response of the endothelium differs between conduit vessels (the aorta) and resistance vessels (mesenteric arteries).[29] Another study[35] on peripheral circulation shows vasodilatation of mesenteric, renal, and iliac arteries from the release of nitric oxide as a result of progesterone infusion.

Conclusion

Today's medical professionals are taught that progesterone is used in hormone replacement therapy to prevent endometrial hyperplasia, to treat infertility, or to support progesterone-deficient pregnancies. However, in this report we have shown that bioidentical progesterone exerts many additional effects throughout the body. Progesterone is critical to ensuring bone health. It offers neuroprotection, contributes to cardiovascular health, assists normal brain development, and provides protection from some types of cancer. The use of bioidentical progesterone may soon be indicated for the treatment of a large population that includes men, women, and (possibly) infants. Synthetic progestins cannot be substituted for many of the favorable actions of bioidentical progesterone.

References

1. Graham C. Physiological action of progesterone in target tissues. *Endocr Rev* 1997;18:502-519.
2. O'Malley BW, Spelsberg TC, Steggles AW. Progesterone-binding components of chick oviduct v. exchange of progesterone-binding capacity from target to non-target tissue chromatins. *J Biol Chem* 1972;247:1368-1374.
3. MacNamara P, Loughrey HC. Progesterone receptor A and B isoform expression in human osteoblasts. *Calcif Tissue Int* 1998;63:39-46.
4. Luo XH, Liao EY. Progesterone differentially regulates the membrane-type matrix metalloproteinase-1(MT1-MMP) compartment of proMMP-2 activation in MG-63 cells. *Horm Metab Res* 2001;33:383-388.
5. Gronowicz GA, McCarthy M-B. Glucocorticoids inhibit the attachment of osteoblasts to bone extracellular matrix proteins and decrease B1-integrin levels. *Endocrinology* 1995;136:598-608.
6. Prior JC. Progesterone as a bone-trophic hormone. *Endocr Rev* 1990;11:386-398.
7. Trotter A, Pohlandt F. The replacement of oestradiol and progesterone in very premature infants. *Ann Med* 2000;32:608-614.
8. Trotter A, Pohlandt F, Bokelmann B. Follow-up examination at the age of 15 months of extremely preterm infants after postnatal estradiol and progesterone replacement. *J Clin Endocrinol Metab* 2001;86:601-603.
9. Shyamala G. Progesterone action in human breast cancer. 1995. Available at: http://www.ucop. edu/srphome/bcrp/progressreport/abstracts/patho/1ib0448.html. Accessed October 17, 2001.
10. Sivaraman L, Conneely, OM, Medina D, et al. p53 is a potential mediator of pregnancy and hormone-induced resistance to mammary carcinogenesis. *Proc Natl Acad Sci USA* 2001;98:12379-12384.
11. Shi-Zhong B, De-Ling Y, Xiu-Hai R, et al. Progesterone induces apoptosis and up-regulation of p53 expression in human ovarian carcinoma cell lines. *Cancer* 1997;79:10.
12. Yu S, Lee M, Shin S, et al. Apoptosis induced by progesterone in human ovarian cancer cell line SNU-840. *J Cell Biochem* 2001;82:445-451.
13. Formby B, Wiley TS. Progesterone inhibits growth and induces apoptosis in breast cancer cells: Inverse effects on Bcl-2 and p53. *Ann Clin Lab Sci* 1998;28:360-369.
14. Formby B, Wiley TS. Bcl-2, surviving and variant CD44 v 7-v10 are downregulat-

ed and p53 is upregulated in breast cancer cells by progesterone: Inhibition of cell growth and induction of apoptosis. *Mol Cell Biochem* 1999;202:53-61.
15. Hofseth L, Raafat A, Osuch JR, et al. Hormone replacement therapy with estrogen or estrogen plus medroxyprogesterone acetate is associated with increased epithelial proliferation in the normal postmenopausal breast. *J Clin Endocrinol Metab* 1999; 84:4559-4565.
16. Foidart JM, Colin C, Denoo X, et al. Estradiol and progesterone regulate the proliferation of human breast epithelial cells. *Fertil Steril* 1998;69:963-969.
17. Trotter A, Maier L, Pohlandt F. Management of the extremely preterm infant: Is the replacement of estradiol and progesterone beneficial? *Paediatr Drugs* 2001;3:629-637.
18. Wagner CK, Nakayama AY, De Vries GJ. Potential role of maternal progesterone in the sexual differentiation of the brain. *Endocrinology* 1998;139: 3658-3661.
19. Baulieu EE, Schumacher M. Neurosteroids, with special reference to the effect of progesterone on myelination in peripheral nerves. *Mult Scler* 1997; 3:105-112.
20. Groswasser Z, Cohen M, Keren O. Female TBI patients recover better than males. *Brain Inj* 1998; 12:805-808.
21. Wright DW, Bauer ME, Hoffman SW, et al. Serum progesterone levels correlate with decreased cerebral edema after traumatic brain injury in male rats. *J Neurotrauma* 2001;18:901-909.
22. Singh M. Ovarian hormones elicit phosphorylation of Akt and extracellular-signal regulated kinase in explants of the cerebral cortex. *Endocrine* 2001; 14:407-415.
23. Nilsen J, Brinton RD. Impact of progestins on estrogen-induced neuroprotection: Synergy by progesterone and 19-norprogesterone and antagonism by medroxyprogesterone acetate. *Endocrinology* 2002;143:205-212.
24. Graham JD, Clarke CL. Physiological action of progesterone in target tissues. *Endocr Rev* 18:502-519.
25. VanDoren MJ, Matthews DB, Janis GC, et al. Neuroactive steroid 3 α-hydroxy-5α-pregnane-20-one modulates electrophysiological and behavioral actions of ethanol. *J Neurosci* 2000;20:1982-1989.
26. Marshall JC, Eagleson CA, McCartney CR. Hypothalamic dysfunction. *Mol Cell Endocrinol* 2001; 183;29-32.
27. Kuttenn F, Gerson M. Hormone replacement therapy of menopause, heart and blood vessels. *Arch Mal Coeur Vaiss* 2001;94:685-689.
28. Sitruk-Ware R. Progestins and cardiovascular risk markers. *Steroids* 2000;65:651-658.
29. Chan HY, Yao X. Different role of endothelium/nitric oxide in 17β-estradiol and progesterone-induced relaxation in rat arteries. *Life Sci* 2001;69: 1609-1617.
30. Crews JK, Khalil RA. Antagonistic effects of 17β-estradiol, progesterone, and testosterone on Ca^{++} entry mechanisms of coronary vasoconstriction. *Arterioscler Thromb Vasc Biol* 1999;19:1034-1040.
31. Mather KJ, Norman EG, Prior JC, et al. Preserved forearm endothelial responses with acute exposure to progesterone: A randomized cross-over trial of 17β-estradiol, progesterone, and 17β-estradiol with progesterone in healthy menopausal women. *J Clin Endocrinol Metab 2000*;85:4644-4649.
32. Lee WS, Harder JA. Progesterone inhibits arterial smooth muscle cell proliferation. *Nat Med* 1997;3: 1005-1008.
33. Karas RH, van Eickels M, Lydon JP, et al. A complex role for the progesterone receptor in the response to vascular injury. *J Clin Invest* 2001;108:611-618.
34. Jiang C, Sarrel PM, Lindsay DC, et al. Progesterone induces endothelium-independent relaxation of rabbit coronary artery in vitro. *Eur J Pharmacol* 1992;211: 163-167.
35. Molinari C, Battaglia A. Effect of progesterone on peripheral blood flow in prepubertal female anesthetized pigs. *J Vasc Res* 2001;38:569-577.

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