The Effects of Progesterone on Glial Cell Line-derived Neurotrophic Factor Secretion from C6 Glioma Cells

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Abstract

Objective(s)
Progesterone is a steroid hormone whose biology has been greatly studied within the confines of reproductive function. In recent years, the neuroprotective effects of progesterone have attracted growing interest. Glial cell line-derived neurotrophic factor (GDNF), is a neurotrophic factor which plays a crucial role in the development and maintenance of distinct sets of central and peripheral neurons. In the present study, we investigated the potential implication of GDNF in the neuroprotective action of progesterone.

Materials and Methods
Cultured rat C6 glioma cells were treated with progesterone (100 nm, 1 µM, and 10 µM) or its vehicle. After 24, 36, 48 and 72 hr, GDNF protein levels were measured in the cell-conditioned media and cell lysates using a GDNF ELISA kit. Cell numbers were determined by a cell-counting assay kit.

Results
Forty-eight hr treatment with progesterone (10 µM) resulted in a significant elevation of GDNF secretion from C6 glioma cells that remained elevated up to 72 hr. The intracellular content of GDNF and cell numbers were not affected by progesterone treatment.

Conclusion
Stimulation of GDNF release from glial cells appears as a potential mechanism through which progesterone exerts its neuroprotective effects.

Keywords: C6 glioma cells, GDNF, Progesterone

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**Introduction**

The female sex steroid, progesterone, is a major gonadal hormone which mainly contributes to the protection of fetus during the gestation period (1). Progesterone, besides its effects on the reproductive and endocrine systems, acts as a neurosteroid in the central nervous system (2-4) and exerts a wide range of actions depending on the target tissue (5-7). This neuroactive steroid has been found to be beneficial as a neuroprotectant in a number of animal species and in several modes of neurological injuries (8, 9). In fact, observing a sex difference in response to an experimentally induced traumatic brain injury was the first evidence for the neuroprotective properties of progesterone. According to Stein, females tend to recover more quickly from traumatic brain injury that may have a hormonal basis (10). He found out that experimentally brain-injured female rats with elevated levels of serum progesterone sustain less neurological damage and recover better than female rats with low progesterone levels at the time of injury. In the peripheral neuropathies, progesterone has been shown to promote the remyelination and axonal regeneration (11). Furthermore, treatment with progesterone restores the expression of molecular markers that characterize motoneurons and promotes proliferation and differentiation of oligodendrocyte progenitors in the experimental spinal cord injury (12, 13). The protective effects of progesterone following cerebral ischemia have also been shown (14, 15). Meanwhile, the mechanism(s) through which progesterone exerts its neuroprotective effects are not clearly defined. There are reports indicating that progesterone protects cultured PC12 cells against the death due to the deprivation of neurotrophic support (16, 17). Neurotrophic factors are proteins that exert survival-promoting and trophic actions on neurons in the peripheral and central nervous systems (18, 19). In recent years, a growing interest has been attracted towards the protective effects of neurotrophic factors in various types of neuronal pathologies (20-23). In this context, an accumulating body of research is dedicated to the synthesis and secretion of neurotrophic factors including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in order to develop novel therapeutic approaches for the treatment of depressive or neurodegenerative disorders (24-32). Considerable efforts devoted to the discovery of novel neurotrophic factors have shown that glial cell line-derived neurotrophic factor (GDNF), a small protein which promotes the survival of many types of neurons, exerts neuroprotective effects (33-36). GDNF mRNA is widely expressed in the peripheral tissues of developing mammalian and avian neurons (37). The most prominent feature of GDNF is its ability to support the survival of dopaminergic and motor neurons which die in the course of Parkinson’s disease or amyotrophic lateral sclerosis (38-42). According to Hisaoka et al., antidepressant drugs increase GDNF release from C6 glioma cells, a rich source of GDNF that may be implicated in their neuroprotective properties (43). This background prompted us to investigate whether progesterone is able to stimulate GDNF secretion from C6 glioma cells.

**Materials and Methods**

**Cell culture and drug treatment**

Rat C6 glioma cell line was obtained from the National Cell Bank of Iran (NCBI, Pasteur Institute of Iran, Tehran, Iran). We used two different culture media: Dulbecco’s Modified Eagle’s Medium (DMEM) and serum-free Opti-MEM (Gibco, UK). All media contained 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma Aldrich, Germany). Incubations were conducted at 37 °C in 5% CO₂ and 95% air. C6 cells were grown in DMEM supplemented with 2 mM/L-glutamine and 5% fetal bovine serum (FBS, Gibco). Cells were seeded into 6-well plates at a density of 4×10⁵/ml in 1 ml of growth medium, allowed to adhere for 24 hr and then medium was replaced with serum-free Opti-MEM containing 0.5% bovine serum albumin (BSA, Gibco). Afterwards, the cells were incubated for 24 hr and the medium was replaced with fresh Opti-MEM and 1 ml of...
0.5% BSA containing progesterone (Sigma Aldrich, Germany) at concentrations of 100 nanomolar (nM), 1 micromolar (μM), and 10 μM (7). Cells were treated with progesterone for 12, 24, 36, 48 and 72 hr (43). Control cultures consisted of the culture media and vehicle of progesterone (ethanol, Sigma). The concentration of ethanol in the culture was less than 0.01%. At the end of each time period, the conditioned medium was aspirated and the GDNF protein level was measured using a GDNF ELISA kit (Chemicon, UK) according to the manufacturer’s instructions. Briefly, 96-well, flat-bottomed plates were coated with anti-GDNF monoclonal antibody and incubated overnight at 4 °C. Samples and standards were incubated at room temperature for 6 hr. The captured GDNF was incubated overnight at 4 °C with chicken anti-human GDNF polyclonal antibody. After washing the plates, horseradish peroxidase-conjugated anti-chicken immunoglobulin Y antibody was added to the plates (100 μl per well) and incubated at room temperature for 2 hr. The plates were washed and the enzyme substrate was added (100 μl per well). Then, the plates were incubated for 15 min at room temperature in the dark. The absorbance at a wavelength of 450 nm was recorded on a microplate reader (Molecular Devices, UK). In order to evaluate whether the release of GDNF was the result of leakage from damaged cells, we measured the amounts of GDNF located within the C6 cells as well as the effect of progesterone on cell numbers. For the measurement of intracellular GDNF content, cells were lysed with 1% NP-40 cell lysis buffer (Sigma) and GDNF protein level was determined by GDNF ELISA kit. Cell numbers were determined using the CCK-8 assay (Cell Counting Kit-8, Roche Diagnostics, Germany). Briefly, after the treatment with different concentrations of progesterone, 10 μl of CCK-8 solution was added to each well in a 96-well plate. After the incubation for 2 hr at 37 °C, the absorbance of the samples at the wavelength of 450 nm was measured by a UV Max kinetic microplate reader (Molecular Devices, UK). In this assay, WST-8 (2-[2-methoxy-4-nitrophenyl]-3-[4-nitrophenyl]-5-[2,4-disulfophenyl]-2H-tetrazolium, monosodium salt) is reduced by dehydrogenases in the cells to give a soluble yellow-coloured product (formazan). The amount of formazan dye generated by the activity of dehydrogenases is directly proportional to the number of living cells.

**Statistics**

The effects of progesterone on GDNF protein levels and C6 glioma cell numbers were analyzed using analysis of variance (ANOVA) followed by Tukey’s post-hoc test. Data are expressed as mean±SEM. The level of significance was set at \( P<0.05 \).

**Results**

**The effects of progesterone on GDNF content of conditioned media**

Forty-eight hr after the treatment with progesterone (10 μM), the secretion of GDNF was significantly increased from the cultured C6 glioma cells into the medium (Figure 1, \( P<0.05 \)). GDNF protein level remained elevated up to 72 hr (Figure 1, \( P<0.01 \)). After the treatment for 24 and 36 hr, progesterone did not affect GDNF secretion as compared with vehicle-treated control groups (Figure 1). Lower concentrations of progesterone did not induce any significant change in GDNF protein content (Figure 1).

**The effects of progesterone on GDNF levels in cell lysates**

Treating the C6 cells with different concentrations of progesterone for 24, 36, 48, and 72 hr did not alter GDNF protein levels in the cell lysates (Figure 2).

**The effects of progesterone on the cell growth of C6 cells**

Treating the C6 cells with progesterone at concentrations ranging from 100 nM to 10 μM in the serum-free conditions had no effect on cell numbers at any time point tested (Figure 3).
Progesterone and GDNF

**Discussion**

Progesterone, in addition to its effects on the reproductive system, has been shown to exert beneficial and neuroprotective effects in the injured central and peripheral nervous systems. There is considerable evidence that progesterone limits tissue damage and improves functional outcome after traumatic brain injury, stroke, spinal cord injury, diabetic neuropathies, and other types of acute neuroinjury in several species (14, 44-50). Meanwhile, the probable mechanisms underlying the neuroprotective effects of progesterone still remain elusive. In the present work, we evaluated the effects of progesterone on GDNF secretion from C6 glioma cells as an in vitro model system. As it is observed in Figure 1, progesterone significantly elevated GDNF release in concentration- and time-dependent manner. Data are presented as mean±SEM (n=6). *P<0.05, **P<0.01 vs. vehicle group.

(nM: nanomolar, µM: micromolar, Prog: progesterone)

![Figure 1](image1.png)

**Figure 1.** The effects of progesterone on GDNF content in cultured C6 cells. Treatment with progesterone resulted in a significant elevation of GDNF protein level in a concentration- and time-dependent manner. Data are presented as mean±SEM (n=6). *P<0.05, **P<0.01 vs. vehicle group.

![Figure 2](image2.png)

**Figure 2.** The effects of progesterone on intracellular GDNF content. GDNF level was not altered by treatment with progesterone at any concentration or time point tested. Data are presented as mean±SEM (n=6)

![Figure 3](image3.png)

**Figure 3.** The effects of progesterone on the cell growth of C6 cells. Treating the cells with progesterone at concentrations ranging from 100 nM to 10 µM for up to 72 h in the serum-free conditions had no effect on cell numbers. Data are presented as mean±SEM (n=6)

As it is observed in Figure 1, progesterone significantly elevated GDNF release in concentration- and time-dependent manner. In parallel, we measured the amounts of GDNF located within the C6 cells in order to evaluate whether the release of GDNF was the result of the leakage from damaged cells. We found that treating the C6 cells with various concentrations of progesterone for up to 72 h did not alter the amount of GDNF present in the cell lysates (Figure 2). Furthermore, treating the cells with progesterone in the serum-free conditions had no effect on the cell numbers (Figure 3), indicating that progesterone had no effect on cell proliferation or cell death. Therefore, these findings indicate that progesterone-induced elevation of GDNF content in the conditioned media is not due to the leakage from damaged cells. If GDNF secretion was simply a consequence of leakage from the cells damaged by progesterone treatment, therefore a decrease in intracellular GDNF levels or the number of cells might have been observed over time, but such significant decreases were not found following progesterone treatment (Figures 2 and 3). In addition, if progesterone-induced secretion of GDNF was due to the leakage of GDNF from damaged cells, a continuous release might have been expected over time, but the time course of GDNF release showed that a significant release was observed only after a
48 hr incubation period but not at earlier time points (Figure 1). Therefore, it appears that enhancement of GDNF secretion by glial cells is implicated, at least in part, in the mechanism of action of progesterone that, in turn, may result in neuroprotection and restoration of neuronal integrity and plasticity. As aforementioned, the probable mechanisms underlying the neuroprotective effects of progesterone have not yet been fully understood, as progesterone does not target a single class of receptors or one cell type (51). In fact, progesterone has manifold actions in the brain, therefore, this neuroactive steroid may alter the expression of as-yet-unidentified gene(s) and proteins involved in the cytotoxic or repair processes. As previously reported, prevention of inflammation (52), excitotoxicity (53), and apoptosis (54, 55), as well as promoting remyelination (56, 57) may be implicated in the neuroprotective action of progesterone. According to Kaur et al, progesterone may exert protective effects through its ability to elicit activation of specific signaling pathways relevant to neuroprotection (58). Regarding the effects of progesterone on neurotrophic factors, progesterone-induced upregulation of BDNF expression has previously been reported (58, 59). Our findings demonstrate that progesterone is able to elevate GDNF secretion from C6 glioma cells (Figure 1). This offers a novel mechanism through which progesterone may exert its neuroprotective effects. In addition, since glial cells are implicated in the development, survival, and metabolism of neuronal cells (60) and modulate the synaptogenesis in the brain (61-63), therefore, progesterone by in vivo stimulation of GDNF secretion from glial cells may be proved to be beneficial for neurodegenerative disorders such as Alzheimer’s disease.

**Conclusion**

Progesterone is able to increase GDNF secretion from glial cells. This, represents a new pathway through which progesterone may protect neurons and restore neuronal integrity and plasticity.

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**References**

1. Di Cosmo A, Paolucci M, Di Cristo C, Botte V, Ciarcia G. Progesterone receptor in the reproductive system of the female of *Octopus vulgaris*: characterization and immunolocalization. Mol Reprod Dev 1998; 50:451–460.
2. Inoue T, Akahira J, Suzuki T, Daranel AD, Kaneko C, Takahashi K, et al. Progesterone production and actions in the human central nervous system and neurogenetic tumors. J Clin Endocrinol Metab 2002; 87:5325–5331.
3. Meffre D, Delespierre B, Gouezou M, Leclerc P, Vinson GP, Schumacher M, et al. The membrane-associated progesterone-binding protein 25-Dx is expressed in brain regions involved in water homeostasis and is up-regulated after traumatic brain injury. J Neurochem 2005; 93:1314–1326.
4. Sakamoto H, Shikimi H, Ukena K, Tsutsui K. Neonatal expression of progesterone receptor isoforms in the cerebellar Purkinje cell in rats. Neurosci Lett 2003; 343:163–166.
5. Reddy DS, O’Malley BW, Rogawski MA. Anxiolytic activity of progesterone in progesterone receptor knockout mice. Neuropharmacology 2005; 48:14–24.
6. Bitran D, Shiek M, McLeod M. Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain GABA receptor a1 receptors. J Neuroendocrinol 1995; 7:171–177.
7. Coughlan T, Gibson C, Murphy S. Progesterone, BDNF and neuroprotection in the injured CNS. Int J Neurosci 2009; 119:1718–1740.
8. Gibson CL, Coomber B, Rathbone J. Is progesterone a candidate neuroprotective factor for treatment following ischemic stroke? Neuroscientist 2009; 15:324-332.
9. Singh M. Progesterone-induced neuroprotection. Endocrine 2006; 29:271-274.
10. Stein DG. Brain damage, sex hormones and recovery: a new role for progesterone and estrogen? Trends Neurosci 2001; 24:386–391.
11. Desarnaud F, Do Thi AN, Brown AM, Greg L, Suter U, Baulieu EE, et al. Progesterone stimulates the activity of the promoters of peripheral myelin protein-22 and protein zero genes in Schwann cells. J Neurochem 1998; 71:1765-1768.
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12. De Nicola AF, Labombarda F, Gonzalez DMC, Gonzalez SL, Garay L, Meyer M, et al. Progesterone neuroprotection in traumatic CNS injury and motoneuron degeneration. Front Neuroendocrinol 2009; 30:173-187.

13. Gonzalez Deniselle MC, López-Costa JJ, Saavedra JP, Pietranera L, Gonzalez SL, Garay L, et al. Progesterone neuroprotection in the Wobbler mouse, a genetic model of spinal cord motor neuron disease. Neurobiol Dis 2002; 11:457-468.

14. Gibson CL, Murphy SP. Progesterone enhances functional recovery after middle cerebral artery occlusion in male mice. J Cereb Blood Flow Metabol 2004; 24:805–813.

15. Sayeed I, Wali B, Stein DG. Progesterone inhibits ischemic brain injury in a rat model of permanent middle cerebral artery occlusion. Res Neurol Neurosci 2007; 25:151–159.

16. Coughlan T, Gibson C, Murphy S. Progesterone, BDNF and neuroprotection in the injured CNS. Int J Neurosci 2009; 119:1718–1740.

17. Akan P, Kizildag S, Ormen M, Genc S, Öktem MA, Fadiloglu M. Pregnenolone protects the PC-12 cell line against amyloid beta peptide toxicity but its sulfate ester does not. Chem Biol Interact 2009; 177:65-70.

18. McKay SE, Purcell AL, Carew TJ. Regulation of synaptic function by neurotrophic factors in vertebrates and invertebrates: implications for development and learning. Learn Mem 1999; 6:193-215.

19. Logan A, Ahmed Z, Baird A, Gonzalez AM, Berry M. Neurotrophic factor synergy is required for neuronal survival and disinhibited axon regeneration after CNS injury. Brain Neurosci Res 2006; 83:50-60.

20. Nomura T, Homnou O, Harada K, Houkin K, Hamada H, Kocsis JD. IV. infusion of brain-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in adult rat. Neuroscience 2005; 136:161-169.

21. Schabitz WR, Steigleder T, Cooper-Kuhn CM, Schwab S, Sommer C, Schneider A, et al. Intravenous brain-derived neurotrophic factor enhances poststroke sensorimotor recovery and stimulates neurogenesis. Stroke 2007; 38: 2165–2172.

22. Zhang Y, Partridge WM. Blood-brain barrier targeting of BDNF improves motor function in rats with middle cerebral artery occlusion. Brain Res 2006; 1111:227–229.

23. Obara Y, Nakahata N. The signaling pathway of neurotrophic factor biosynthesis. Drug News Perspect 2002; 15:290-298.

24. Hassanzadeh P, Rahimpour S. The cannabinergic system is implicated in the upregulation of central NGF protein by psychotropic drugs. Psychopharmacology 2011; 215:129–141.

25. Hassanzadeh P, Hassanzadeh A. Involvement of the neurotrophin and cannabinoid systems in the mechanisms of action of neurokinin receptor antagonists. Eur Neuropsychopharmacol 2011; 21:905-917.

26. Hassanzadeh P, Hassanzadeh A. Effects of different psychotropic agents on the central nerve growth factor protein. Iran J Basic Med Sci 2010; 13:202-209.

27. Hassanzadeh P. The endocannabinoid system: critical for the neurotrophic action of psychotropic drugs. Biomed Rev 2010; 21:31-46.

28. Takahashi M, Shirakawa O, Toyooka K, Kitamura N, Hashimoto T, Maeda K, et al. Abnormal expression of brain-derived neurotrophic factor and receptor in the corticolineic system of schizophrenic patients. Mol Psychiatry 2000; 5:293-300.

29. Chlan-Fourney J, Ashe P, Nylen K, Juorio AV, Li XM. Differential regulation of hippocampal BDNF mRNA by typical and atypical antipsychotic administration. Brain Res 2002; 954:11-20.

30. Xu H, Qing H, Lu W, Keegan D, Richardson JS, Chlan-Fourney J, et al. Quetiapine attenuates the immobilization stress-induced decrease of brain-derived neurotrophic factor expression in rat hippocampus. Neurosci Lett 2002; 321:65-68.

31. Bai O, Chlan-Fourney J, Bowen R, Keegan D, Li XM. Expression of brain-derived mRNA in rat hippocampus after treatment with antipsychotic drugs. J Neurosci Res 2003; 71:127-131.

32. Sawada H, Ibi M, Kihara T, Urushitani M, Nakanishi M, Akaite A, et al. Neuroprotective mechanism of glial cell line-derived neurotrophic factor in mesencephalic neurons. J Neurochem 2000; 74:1175-1184.

33. Lara J, Kusano K, House S, Gainer H. Interactions of cyclic adenosine monophosphate, brain-derived neurotrophic factor, and glial cell line-derived neurotrophic factor treatment on the survival and growth of postnatal mesencephalic dopamine neurons in vitro. Exp Neurol 2003; 180:32-45.

34. Hauck SM, Kinkl N, Deeg CA, Swiatek-de Lange M, Schoffmann S, Ueffing M. GDNF family ligands trigger indirect neuroprotective signaling in retinal glial cells. Mol Cell Biol 2006; 26:2746-2757.

35. Chena B, Gaoa XQ, Yanga CX, Tana SK, Suna ZL, Yamb NH, et al. Neuroprotective effect of grafting GDNF gene-modified neural stem cells on cerebral ischemia in rats. Brain Res 2009; 1284:1-11.
37. Trupp M, Ryden M, Jornvall H, Funakoshi H, Timmusk T, Arenas E, et al. Peripheral expression and biological activities of GDNF, a new neurotrophic factor for avian and mammalian peripheral neurons. J Cell Biol 1995; 130:137-148.
38. Lin LF, Doherty DH, Lile JD, Bektash S, Collins F. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 1993; 260:1130–1132.
39. Ugarte SD, Lin E, Klann E, Zigmund MJ, Perez RG. Effects of GDNF on 6-OHDA-induced death on a dopaminergic cell line: modulation by inhibitors of PI3 kinase and MEK. J Neurosci Res 2003; 73:105-112.
40. Tomac A, Lindqvist E, Lin LF, Ogren SO, Young D, Hoffer BJ, et al. Protection and repair of the nigrostriatal dopaminergic system by GDNF in vivo. Nature 1995; 373:335-339.
41. Oppenheim RW, Houenou LJ, Johnson JE, Lin LH, Linxi LI, Lo AC, et al. Developing motor neurons rescued from programmed and axotomy-induced cell death by GDNF. Nature 1995; 373: 344-346.
42. Gash DM, Zhang Z, Ovadia A, Cass WA, Yi A, Simmerman L, et al. Functional recovery in parkinsonian monkeys treated with GDNF. Nature 1996; 380: 252-255.
43. Hisaoka K, Nishida A, Koda T, Miyata M, Zensho H, Morinobu S, et al. Antidepressant drug treatments induce glial cell line-derived neurotrophic factor (GDNF) synthesis and release in rat C6 gliblastoma cells. J Neurochem 2001; 79:25-34.
44. Guo Q, Sayeed I, Baronne LM, Hoffman SW, Guennoun R, Stein DG. Progesterone administration modulates AQP4 expression and edema after traumatic brain injury in male rats. Exp Neurol 2006; 198:469–478.
45. He J, Evans CO, Hoffman SW, Oyesiku NM, Stein DG. Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. Exp Neurol 2004; 189:404–412.
46. Leonelli E, Bianchi R, Cavaletti G, Caruso D, Crippa D, Garcia-Segura LM, et al. Progesterone and its derivatives are neuroprotective agents in experimental diabetic neuropathy: a multimodal analysis. Neuroscience 2007; 144: 1293–1304.
47. O’Connor CA, Cernak I, Vink R. Both estrogen and progesterone attenuate edema formation following diffuse traumatic brain injury in rats. Brain Res 2005; 1062:171–174.
48. Pettus EH, Wright DW, Stein DG, Hoffman SW. Progesterone treatment inhibits the inflammatory agents that accompany traumatic brain injury. Brain Res 2005; 1049:112–119.
49. Wright DW, Bauer ME, Hoffman SW, Stein DG. Serum progesterone levels correlate with decreased cerebral edema after traumatic brain injury in male rats. J Neurotrauma 2001; 18: 901–909.
50. Kumon KSC, Tompkins P, Stevens A, Sakaki S, Loftus CM. Neuroprotective effect of posts ischemic administration of progesterone in spontaneously hypertensive rats with focal cerebral ischemia. J Neurosurg 2000; 92:848–852.
51. Stein DG. Progesterone exerts neuroprotective effects after brain injury. Brain Res Rev 2008; 57:386–397.
52. Gibson CL, Constantini D, Prior MJ, Bath PM, Murphy SP. Progesterone suppresses the inflammatory response and nitric oxide synthase-2 expression following cerebral ischemia. Exp Neurol 2005; 193:522–530.
53. Stein DG, Wright DW, Kellermann AL. Does progesterone have neuroprotective properties? Ann Emerg Med 2008; 51:164-172.
54. Djebaili M, Guo Q, Pettus EH, Hoffman SW, Stein DG. The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. J Neurotrauma 2005; 22:106–118.
55. Djebaili M, Hoffman SW, Stein DG. Allopregnanolone and progesterone decrease cell death and cognitive deficits after a contusion of the rat pre-frontal cortex. Neuroscience 2004; 123:349–359.
56. De Nicola AF, Gonzalez SL, Labombarda F, Deniselle MC, Garay L, Guennoun R, et al. Progesterone treatment of spinal cord injury: effects on receptors, neuroproteins, and myelination. J Mol Neurosci 2006; 28:3–15.
57. Labombarda F, Gonzalez S, Gonzalez Deniselle MC, Garay L, Guennoun R, Schumacher M, et al. Progesterone increases the expression of myelin basic protein and the number of cells showing NG2 immunostaining in the lesioned spinal cord. J Neurotrauma 2006; 23:181–192.
58. Kaur P, Jodhka PK, Underwood WA, Bowles CA, de Fiebre NEC, de Fiebre CM, et al. Progesterone increases BDNF expression and protects against glutamate toxicity in a MAPK- and PI3-K-dependent manner in cerebral cortical explants. J Neurosci Res 2005; 85:2441–2449.
59. Gonzalez SL, Labombarda F, Deniselle MC, Mougel A, Guennoun R, Schumacher M, et al. Progesterone neuroprotection in spinal cord trauma involves up regulation of brain-derived neurotrophic factor in motoneurons. J Steroid Biochem Mol Biol 2005; 94:143–149.
60. Freeman MR. Sculpting the nervous system: glial control of neuronal development. Curr Opin Neurobiol 2006; 16:119–125.
61. Haydon PG. GLIA: Listening and talking to the synapse. Nature Rev Neurosci 2001; 2: 185-193.
62. Slezak M, Pfrieger FW. New roles for astrocytes: Regulation of CNS synaptogenesis. Trend Neurosci 2003; 26:531-535.
63. Slezak M, Pfrieger FW, Soltys Z. Synaptic plasticity, astrocytes and morphological homeostasis. J Physiol 2006; 99:84–91.