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## PROGESTERONE EXERTS NEUROPROTECTIVE EFFECTS AFTER BRAIN INJURY

**Donald G. Stein, Ph.D.**

*Brain Research Laboratory, Department of Emergency Medicine, Emory University, Atlanta, GA, USA*

### Abstract

Progesterone, although still widely considered primarily a sex hormone, is an important agent affecting many central nervous system functions. This review assesses recent, primarily *in vivo*, evidence that progesterone can play an important role in promoting and enhancing repair after traumatic brain injury and stroke. Although many of its specific actions on neuroplasticity remain to be discovered, there is growing evidence that this hormone may be a safe and effective treatment for traumatic brain injury and other neural disorders in humans.

### Introduction

Progesterone exerts a wide range of actions, depending on the target tissue. Its effects on the reproductive and endocrine systems are well known, but it also functions as a neurosteroid in the central nervous system (CNS). Progesterone is naturally present in the brains of men and women at similar levels (Baulieu and Robel, 1990; Coughlan et al., 2005; Guerra-Araiza et al., 2002; Inoue et al., 2002; Meffre et al., 2005; Reddy et al., 2005; Sakamoto et al., 2003). Sex hormones are known to influence neuronal differentiation during fetal development. In adults, progesterone affects neuronal function by modulating gene transcription and cellular activity through classical intracellular receptors abundant in the CNS (Auger and De Vries, 2002; Blaustein, 2003; Coughlan et al., 2005; Dufourny and Skinner, 2003; Ghomari et al., 2005; Gruber and Huber, 2003; Labombarda et al., 2003; Lonstein and Blaustein, 2004; Mani, 2006; Ozawa, 2005; Pierson et al., 2005). Progesterone, or its metabolite allopregnanolone, binds to a variety of intracellular receptors, including GABAA (Lambert et al., 2003; Pierson et al., 2005; Smith and Gong, 2005), the Sigma receptor (Baulieu, 1998; Maurice et al., 2001; Monnet and Maurice, 2006), 25-Dx (Meffre et al., 2005), and potentially others. It may also exert some of its effects through non-receptor pathways (Vanlandingham et al., 2006).

Laboratories around the world have reported that progesterone limits tissue damage and improves functional outcome after blunt traumatic brain injury (TBI), stroke, spinal cord injury, diabetic neuropathies, and other types of acute neuroinjury in several species. Progesterone appears to protect or rebuild the blood-brain barrier (BBB), reduce cerebral edema, down-regulate the inflammatory cascade, and decrease apoptosis (Guo et al., 2006; He et al., 2004a; Leonelli et al., 2007; O'Connor et al., 2005; Pettus et al., 2005; Roof et al., 1997; Stein,

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Corresponding author: Donald G. Stein, Ph.D., Asa G. Candler Professor of Emergency Medicine, 1365 B Clifton Rd., N.E., Suite 5100, Emory University, Atlanta, GA 30322, 404 712 9704, 404 727 2388 fax, E-mail: dstei04@emory.edu.

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2005; Wright et al., 2001). All these actions are plausible mechanisms for progesterone's neuroprotective effects.

## What Is the Problem?

Despite decades of effort, scientists have failed to identify a pharmacological agent that consistently improves outcomes following TBI (Alderson and Roberts, 2005; Brain Trauma Foundation, 1996; Cochrane Collection, 2005; Fan et al., 1996; Roberts et al., 1998). In 1993, the Brain Trauma Foundation convened an international task force to develop evidence-based guidelines for treatment of TBI (Brain Trauma Foundation, 1996). At that time, with the possible exception of mannitol and barbiturates, no effective agent had been identified. Later, Roberts and colleagues reviewed five treatments for TBI (hyperventilation, mannitol, CSF drainage, barbiturates, and corticosteroids) and noted that when studies were restricted to those with proper controls, no agent was effective for decreasing morbidity and mortality following TBI (Roberts et al., 1998; Roberts et al., 2004).

No new pharmacotherapy for TBI has entered clinical practice in over 30 years (Roberts et al., 1998). Glucocorticoids, once a mainstay of TBI treatment, are now known to be harmful (Alderson and Roberts, 2000; Gomes et al., 2005; Vink and Van Den Heuvel, 2004; Watson et al., 2004). The Corticosteroids After Significant Head Injury (CRASH) trial, with more than 10,000 subjects in 40 countries, was halted because the treatment group had a significantly higher mortality rate than the control group (Edwards et al., 2005; Roberts et al., 2004). An NIH-funded clinical trial of magnesium sulfate was terminated for the same reason (see Temkin et al., 2007, for details) (Temkin et al., 2007). Hypothermia is useful for treating global ischemia, and has produced favorable effects in some brain-injured subjects. However, it may be harmful to patients over 45 (Alderson et al., 2004; Clifton et al., 1993; Clifton et al., 2001), and the treatment is still considered investigational (Davies, 2005; Pemberton and Dinsmore, 2003; Seppelt, 2005).

## A New Therapeutic Candidate: Progesterone

Discovery of progesterone's neuroprotective properties began with the observation of a gender difference in response to experimentally-induced TBI. Noting anecdotal reports that females tend to recover better than males following a TBI, Stein and co-workers investigated the possibility that this effect might have a hormonal basis (Attella et al., 1987; Stein, 2001). This group conducted a series of studies to determine whether experimentally brain-injured female rats with elevated levels of serum progesterone sustain less neurological damage and recover better than females with low or no progesterone at the time of injury.

Three groups of mature rats were used—males, cycling females in proestrus (when endogenous levels of progesterone are at their nadir and estrogen is high), and pseudopregnant females (with high circulating levels of progesterone and comparatively lower levels of estrogen) (Roof et al., 1993a). Pseudopregnancy was induced by mild mechanical stimulation of the cervix. Animals then received a calibrated contusion to the medial frontal cortex (MFC). Twenty-four hours later, the animals were killed, and tissue-punch samples of brain taken from the lesion area. Same-brain control samples were obtained from the rat's posterior parietal cortex, an area remote from the injury. Blinded analysis of wet-to-dry tissue weights of these samples revealed that female rats in proestrus developed significantly less brain edema than male rats. However, pseudopregnant females developed almost no post-injury cerebral edema.

Since high endogenous progesterone had salutary effects, a second experiment tested whether exogenous progesterone was beneficial (Roof et al., 1993a; Roof et al., 1993b; Roof et al., 1994). Progesterone (4mg/kg) was given by subcutaneous injection one hour after bilateral MFC contusion to male rats and female rats in proestrus. The dose was repeated by

intraperitoneal injections at 6, 24, and 48 hours after injury. Control animals received the same injury, but were injected with vehicle rather than progesterone. Brain samples were obtained at 72 hours post-injury. Analysis of wet-to-dry tissue weights revealed that both the male and female animals treated with progesterone developed significantly less cerebral edema than controls (Roof et al., 1993a) and had significantly smaller lesions and less secondary neuronal loss in the thalamus. Thus, systemic injections of progesterone reduced cerebral edema and had a neuroprotective effect in the damaged adult CNS (see also Betz, Coester 1990a; Betz, Coester, 1990b for early research in this area) (Betz and Coester, 1990a; Betz and Coester, 1990b).

Because increased intracranial pressure from cerebral edema can kill nerve cells, the sooner edema is controlled, the better. To determine how quickly cerebral edema develops and can be resolved with treatment in brain-injured rats, a controlled MFC contusion was given to male and female rats and wet-to-dry brain tissue weights were taken at various times post-injury. Cerebral edema developed in rats within two hours of injury, peaked at 24 hours, and decreased by 7 days. Progesterone given one hour after injury reduced the level of cerebral edema at 3 days to that normally seen at 7 (Roof et al., 1996).

To determine the therapeutic window for progesterone, groups of contused male and female rats were given a first injection of progesterone (4mg/kg) or vehicle at 2, 6, 24, or 48 hours post-injury. The extent of cerebral edema in all animals was assessed 3 days post-injury. As expected, results were best if treatment was initiated within 2 hours of injury. However, significant reductions in cerebral edema were achieved when treatment was delayed as long as 24 hours post-injury (Roof et al., 1996).

Using the same injury model, Wright et al. administered different doses of progesterone beginning immediately post-injury. Within 1–24 hours after treatment, blood was drawn to determine each animal's progesterone level, and brains harvested for analysis. An inverse correlation was noted between measured serum progesterone concentrations and the degree of cerebral edema that developed after TBI. The higher the circulating level of progesterone, the lower the degree of cerebral edema (Wright et al., 2001).

Although this study seems to suggest that more progesterone is better, there appears to be an upper limit for dosage. Goss et al. (Goss et al., 2003) reported that administering relatively high doses of progesterone (32 mg/kg, first SC, then IP) was ineffective. However, the investigators reported that lower doses (8–16/mg/kg) produced beneficial effects on cognitive performance and reduction of inflammatory factors. Such U-shaped functions in dose-response studies are not uncommon, but they are difficult to explain.

To examine progesterone's effects on functional outcomes after TBI, two groups of adult male rats were given bilateral MFC contusions or sham surgery. Systemic injections of progesterone (4mg/kg) or vehicle were administered at 1, 6, 24, 48, 72, 96, and 120 hours post-injury. Behavioral testing with a Morris water maze began 7 days post-injury and continued for 10 days. Not surprisingly, brain-injured males receiving vehicle performed poorly compared to sham-surgery counterparts given either progesterone or vehicle. However, the rats with TBI given progesterone performed significantly better on post-injury testing than placebo controls and almost as well as sham-surgery controls (Roof, 1994). These results were confirmed by Shear et al. (Shear et al., 2002), who found that while 3 days of post-injury systemic progesterone (4mg/kg) could attenuate edema, 5 days of similar treatment were required to reduce neuropathology and obtain more complete functional recovery on sensory neglect and spatial learning tasks.

One issue that has been raised is whether abrupt termination of progesterone after relatively high doses could be detrimental. Progesterone withdrawal syndrome is known to produce

vasomotor and depressive symptoms as well as increases in anxiety, among other effects (Gulinello et al., 2003; Ockene et al., 2005; Stoffel and Craft, 2004). To examine whether progesterone withdrawal could affect its effectiveness in ameliorating TBI outcome, Cutler et al. (Cutler et al., 2005) compared a regimen of gradually tapering doses to one that ended treatment abruptly. One group of brain-injured rats was given daily injections of progesterone at 16mg/kg/day for 5 days, followed by 2 days of progressively halved doses. The second group received 7 days of treatment, which was then abruptly terminated. A third group received vehicle only. Compared to the tapered dose group, the acute withdrawal group developed greater levels of anxiety and expressed higher levels of inflammatory factors and tissue markers of apoptosis (TNF $\alpha$ , cFos, caspase-3 and NF $\kappa$ B, etc.). However, treated animals in both groups achieved better functional and biochemical outcomes than controls receiving only vehicle (Cutler et al.). Thus it now appears that progesterone efficacy can be enhanced by gradually reducing the dose rather than suddenly withdrawing the treatment.

Experimental fluid percussion injury produces a more diffuse type of CNS damage, and is thought to be a model of head injury in humans. Bramlett and Dietrich (Bramlett and Dietrich, 2001) compared male and ovariectomized and intact female rats after fluid percussion injury. Three days post-injury, the animals were sacrificed and their brains examined. On average, brain-injured females had smaller cortical contusions than brain-injured males. Ovariectomized females had injuries similar to males. This study demonstrates that the presence or absence of hormones has an effect on histopathological outcome in this model of injury.

Using the brain injury model described previously (Roof, 1994), progesterone administered one hour after injury has been shown to be superior to equivalent doses of methylprednisolone for reducing edema (Fritts, 1996; Konen, 1997; Roof et al., 1996). One possible explanation is that the steroids exert different effects on cellular excitotoxicity. Methylprednisolone increases glutamate toxicity in the brain by potentiating NMDA response and attenuating GABA response (Foroutan et al., 1996; Uhler et al., 1994). Progesterone reduces excitotoxicity by decreasing the effects of glutamate and boosting the effects of GABA (Baulieu and Schumacher, 1997; Bitran et al., 1995; Foroutan et al., 1996).

## Progesterone in other CNS injury models

Growing evidence indicates that progesterone affords protection from other forms of neuroinjury as well. Asbury and colleagues reported that administering progesterone to rats after bilateral MFC aspiration reduces neuronal loss in the mediodorsal thalamic nucleus and the striatum compared to controls (Asbury et al., 1998). In a rat model of penetrating brain injury, progesterone decreased accumulation of astrocytes in proximity to the injury (Garcia-Estrada et al., 1993; Garcia-Estrada et al., 1999). Recently, O'Connor et al. (O'Connor et al., 2007) created diffuse head injury by dropping a 450g weight onto the exposed skulls of male and female rats. They followed this with 9 days of daily progesterone injections, behavioral testing on the rotorod, and the Barnes maze test for cognitive performance. Both male and ovariectomized (removing the source of estrogen) female progesterone-treated animals showed marked improvement on the rotorod task at every time point and recovered to pre-injury levels by day 3 of testing. Both the males and females showed improved cognitive outcomes. Examination of the brain tissue showed a reduction in neuronal loss and axonal injury as measured by immunostaining—thus confirming the relationship between neuronal/axonal sparing and behavioral recovery in this injury model.

Several groups have reported that progesterone exerts beneficial effects in spinal cord injury, including enhanced remyelination and improved motor function (Baulieu, 1997; Jung-Testas et al., 1994; Jung-Testas and Baulieu, 1998; Koenig et al., 1995; Schumacher and Baulieu,

1995; Schumacher et al., 1996). Using a laminectomy-plus-impact contusion model of spinal cord injury and then testing for functional recovery, Thomas and colleagues (Thomas et al., 1999) randomly administered progesterone or vehicle to adult male rats, then conducted weekly tests for six weeks using the well-validated Basso-Beatty-Bresnehan (BBB) locomotor rating scale. Animals given progesterone achieved significantly better BBB scores than those given vehicle.

Examination of the spinal cords of these animals revealed that progesterone-treated rats had less white matter damage in the area surrounding the injury site than vehicle-treated controls (De Nicola et al., 2006; Gonzalez et al., 2005; Labombarda et al., 2002; Labombarda et al., 2006).

Although several studies on spinal cord or peripheral nerve injury followed by progesterone treatments have found beneficial results, one recent study has failed to do so (Fee et al., 2007). Male and female rats received spinal cord contusion injuries followed by 5 or 14 days of either 8 mg/kg or 16 mg/kg of progesterone administered in DMSO or beta-cyclodextrin. With 5 days of treatment the investigators found no differences in long-term outcome of locomotor function and there was some indication of increasing loss of gray matter in the cord with the higher doses of progesterone. With 14 days of treatment greater sparing was observed in the gray matter compared to vehicle controls. No benefits were seen in functional outcomes. These findings are difficult to reconcile with reports showing more consistent remyelination and better functional outcomes. No molecular measures were taken to determine whether any inflammatory markers for CNS injury were affected by their treatments so it is difficult to determine the reasons for their failure to find a salutary effect of the progesterone treatments.

There is now evidence that progesterone and some of its metabolites may be useful as a treatment for diabetic neuropathy (Leonelli et al., 2007). The authors used streptozotocin to induce diabetic neuropathy in male rats and then exposed them to one month of progesterone or its metabolites DHP and THP. The treatments appeared to sustain nerve conduction velocity, restore skin innervation and maintain sensitivity to thermal stimulation, all of which are substantially decreased by diabetes-induced chronic neuropathy.

### **Progesterone and stroke**

In the mid-1990's, a group of researchers studied whether progesterone could reduce the consequences of transient cerebral ischemia in a temporary occlusion model. Each animal's middle cerebral artery was occluded for several hours, then reperfusion was permitted. Jiang et al. (Jiang et al., 1996) administered systemic progesterone by injection at a dose of 4mg/kg in DMSO to male adult rats either immediately prior to middle cerebral artery occlusion (MCAO) or two hours after reperfusion. Progesterone resulted in a significantly smaller cortical infarct volumes, less weight loss, and better neurologic outcomes whether it was administered pre- or post-injury. Other studies replicated these findings. Progesterone treatment improved neurological outcomes and decreased cortical infarct size in both normotensive (Chen et al., 1999) and spontaneously hypertensive male rats (Kumon et al., 2000).

In a 4-vessel cerebro-vascular occlusion ischemic model, investigators reported that compared to vehicle, 4 intravenous doses (8mg/kg) of progesterone at 2, 6, and 24 hours after reperfusion eliminated ventriculomegaly and significantly reduced loss of pyramidal neurons in the CA1 and CA2 fields of the dorsal hippocampus when measured at 21 days survival. Compared to rats given progesterone, vehicle-treated animals had a twofold increase in ventricular dilation and cerebral shrinkage (Morali et al., 2005).



Sayeed et al. recently determined that both progesterone and its metabolite allopregnanolone were beneficial treatments for stroke caused by temporary or permanent MCAO (Sayeed et al., 2006; Sayeed, 2007). Three days of systemic treatment with either agent resulted in significantly smaller necrotic infarcts and lower levels of cerebral edema compared to vehicle-treated controls. Although progesterone produced favorable effects, allopregnanolone was even better for stroke at half the dose of progesterone. These data suggest progesterone's effects in ischemic brain tissue may be expressed through its metabolite allopregnanolone. However, dose-response studies are needed to determine whether allopregnanolone is in fact more effective than progesterone as a treatment for ischemic stroke.

While most studies have used male rats, there have been reports of progesterone's benefit in female rats subjected to ischemic stroke. Alkayed et al. (Alkayed et al., 2000) used slow-release implants of progesterone or estrogen over 7 days to see whether 16-month-old reproductively senescent females would show smaller cerebral infarcts after MCAO followed by 22 hours of reperfusion. Infarct size did not differ between male age-matched cohorts and the non-treated females, but application of either progesterone or estrogen significantly reduced the cortical infarcts. Only the estrogen treatment appeared to reduce the size of the injury in the striatum. Murphy et al. (Murphy et al., 2002) examined infarction after 2 hours of MCAO followed by reperfusion in ovariectomized rats given various doses of progesterone before injury or both pre-and post- ischemia. The resulting cortical infarctions were significantly reduced in the progesterone-treated animals compared to those given vehicle. As noted above, these results were recently confirmed by O'Connor and colleagues following diffuse axonal injury in both male and female ovariectomized rats (O'Connor et al., 2007).

Most stroke studies have reported positive findings, but some have not. Murphy et al. (Murphy et al., 2000) found that progesterone exacerbated the size of the ischemic injury in progesterone-deficient ovariectomized female rats. Toung and colleagues (Toung et al., 2004) pretreated older female rats with high doses of estrogen and progesterone for sustained periods, then, unlike the Cutler et al. study (Cutler et al., 2005), terminated treatment without tapering once the animals were past the peri-ischemic period. This treatment produced limited benefit in reducing cortical infarcts and no effect on striatal damage. These seemingly discrepant findings may be explained by their experimental designs. In the first case, the female rats used were reproductively senescent and thus may have been less sensitive to the replacement progesterone. In the second study, lack of tapering of treatment may explain the reduction of benefit, an effect noted by Cutler and colleagues in a brain injury model [31]. A recent experiment by Wagner and colleagues (Wagner et al., 2004) found that varying the estrous cycle did not affect TBI outcome of the females, and gender had no effect on spatial learning performance in a water maze. However, the females did do better than males on several motor performance tasks, indicating the task-dependent benefits of hormones after brain injury. Here it is worth emphasizing that regardless of stage of estrus, young adult females have endogenously higher levels of progesterone than males.

## **Progesterone is neuroprotective in several species**

### **Mice**

Gibson and colleagues (Gibson and Murphy, 2004) performed MCAO in a group of male mice, then randomly administered IP injections of 8 mg/kg progesterone in DMSO, or vehicle only. Outcomes were assessed 48 hours after injury. Compared to vehicle-treated controls, progesterone-treated animals achieved a higher rate of survival and better motor function scores on rotorod testing, and performed almost as well as sham surgery controls on a water maze test.

## Cats

Cervantes and colleagues (Cervantes et al., 2002) administered progesterone to ovariectomized cats at 10 mg/kg/day immediately before and for 7 days after hemispheric global ischemia. Control animals received vehicle alone. Cardiac arrest was precipitated for 15 minutes, followed by reanimation. Fourteen days after this global CNS insult, the animals' brains were examined. Microscopic analysis revealed that progesterone-treated cats had more sparing of striatal neurons than vehicle-treated animals and 21–49% less neuronal loss. Behavioral tests were not reported.

## Rabbits

Chavez-Delgado et al. (Chavez-Delgado et al., 2003) damaged the facial nerves of rabbits to study whether subcutaneous implantation of progesterone in a biodegradable prosthesis (Chitosan) aids in nerve regeneration. Tissue measures of regeneration were made under light microscopy 45 days after the implantation. Animals treated with progesterone had more nerve fibers in the proximal and distal facial nerve stumps compared to controls (Chavez-Delgado et al., 2003).

Numerous animal studies in four species by a variety of laboratory groups have reported that progesterone confers neuroprotection in several types of neuroinjury, including blunt trauma, fluid percussion, cortical aspiration, spinal cord injury, stroke, global ischemia and peripheral injury. In most cases, treatment was initiated shortly after the experimental injury and continued for a relatively short period of time (1–7 days). Very high doses of progesterone (30mg/kg and above) may not exert positive effects, and one study suggests that abrupt withdrawal of progesterone treatment may produce deleterious effects. This withdrawal effect may explain why a few researchers failed to achieve favorable results (Fee et al., 2007).

## Clinical Results in the Treatment of Traumatic Brain Injury

The single clinical trial investigating progesterone to date was limited to closed head blunt trauma with moderate to severe damage in 100 male and female patients (Wright et al., 2007). Over 70% of the subjects were in the "severe" category. Patients received state-of-the-art emergency treatment plus vehicle or progesterone. The progesterone group received three days of post-injury continuous intravenous drip. At 30 days post-injury the severely injured patients showed a statistically significant reduction in mortality compared to those receiving vehicle (i.e., 13.4 versus 33.6%). Moderate TBI/progesterone patients had significantly better functional outcome (Disability Rating Scale) scores than the placebo group. The promising results from this small trial must be confirmed in a much larger, multi-center trial now being planned. Nonetheless, the findings represent the first successful clinical trial for treating TBI in the last 40 years. Whether progesterone would be beneficial in the clinical treatment of stroke still needs to be examined.

## Progesterone's Neuroprotective Mechanisms

### Edema

Brain swelling accounts for a substantial proportion of the morbidity and mortality associated with TBI, brain tumors, and stroke (Marmarou, 2004; Papadopoulos et al., 2002). Inflammatory reactions initiated by TBI and stroke trigger the breakdown of brain tissue, which in turn leads to cerebral edema and further cell loss. There are basically two phases of edema attributed to brain injury, although there is some contention that cytotoxic edema is the more likely following TBI (Marmarou et al., 2006a; Marmarou et al., 2006b). Vasogenic edema is caused by disruption of the BBB, which allows plasma fluid to enter the brain parenchyma, causing increases in intracranial pressure and further neuronal cell loss. A second phase of edema,

cytotoxic, is caused by the accumulation of fluid *inside* neurons and reactive astrocytes. This causes cells to disrupt and release additional toxic agents into the brain parenchyma, producing a cycle of secondary cell death. Progesterone has been shown to reduce both vasogenic and cytotoxic edema after TBI (Roof et al., 1996; Roof, 1992) and MCAO stroke (Gibson and Murphy, 2004; Gibson et al., 2005; Kumon et al., 2000; Sayeed et al., 2006; Sayeed, 2007). As noted above, in one experimental model serum levels of progesterone were inversely correlated with the degree of edema after TBI (Wright et al., 2001). The mechanisms of water removal by progesterone treatment are not yet fully understood, but there are some interesting clues.

Recently, Meffre et al. (Meffre et al., 2005) used intact and cortically contused male and pseudo-pregnant female rats to examine the role of a membrane progesterone binding protein, 25-Dx, which is speculated to play a role in progesterone-mediated osmoregulation. 25-Dx is “especially abundant” in those regions of the brain proximal to cerebral spinal fluid, including regions of the hypothalamus associated with osmoregulation. After TBI in both male and female rats, 25-Dx expression was *increased* in these regions compared to controls, and was specifically co-localized (with immunocytochemical markers for the protein) to reactive astrocytes around the cortical injury site. 25-Dx expression was higher in the subcommisural organ and choroid plexus of females than males. This may be one reason why females with higher levels of circulating progesterone have less edema than males, although both can benefit from exogenous progesterone (Attella et al., 1987). The authors suggest that this is not just an arcane point because progesterone, through its actions on the 25-Dx membrane receptor, may significantly increase the expression of vasopressin (for fuller review of 25-Dx mechanisms and actions, see Guennoun et al., this volume).

We are now learning more about aquaporins (AQP), a class of water channel membrane proteins that can modulate cerebral edema. AQP-4, located in the end-feet of astrocytes and microglia, can act as an osmosensor and control water drainage into the ventricles of the brain (Kleindienst et al., 2006). Guo et al. (Guo et al., 2006) found that, following cortical TBI, there was a substantial increase in edema, especially within the first 24 hours. This was significantly decreased by only two injections of progesterone. Progesterone-treated rats had less edema in the zone of the injury, which correlated with lowered expression of AQP-4 around the lesion site and in tissue surrounding the lateral ventricles.

### Lipid peroxidation and oxidative stress

Although progesterone does not have the characteristic structure of an antioxidant, high levels of the hormone are effective in reducing free radical damage (Goodman et al., 1996; Roof, 1997; Subramanian et al., 1993; Vedder et al., 1999). Pregnancy can reduce lipid peroxidation in brain homogenates and mitochondria (Goodman et al., 1996; Roof et al., 1997; Roof, 1997; Subramanian et al., 1993; Vedder et al., 1999). Progesterone administration reduces lipid peroxidation in different types of *in vitro*, free-radical-generating systems in a dose-dependent manner (Shimamura et al., 1995; Subramanian et al., 1993; Vedder et al., 1999). It also increases levels of mitochondrial glutathione, a critical free radical scavenger (Subramanian et al., 1993); protects mitochondrial function in neural cells *in vitro* after mechanical stretch injury (Malcolm, 2000); and down-regulates injury-induced increases in manganese superoxide dismutase (Cargill, 1999). Progesterone treatment results in less nitrite, superoxide, and hydrogen peroxide generated by cultured macrophages in response to cytokines (Chao, 1994). Macrophages are known to be very active between 48 hours and seven days after TBI, and a reduction of these reactive cells may reduce secondary damage to neurons (Fulop, 1992; Holmin et al., 1995; Soares et al., 1995a; Soares et al., 1995b). Following cortical contusions, rats given progesterone had significantly less 8-isoprostane, a vasoconstrictive



free-radical-generated prostaglandin, than untreated controls 24 and 48 hours post-injury (Roof et al., 1997; Roof, 1997).

Progesterone also seems to be beneficial in preventing mitochondrial dysfunction that results in loss of hippocampal cells after a controlled cortical contusion (Robertson et al., 2006). Female rats given progesterone supplements via silastic implants had no loss of mitochondrial oxygen consumption and there was virtually complete preservation of hippocampal neurons in the CA1 and CA3 subfields of the hippocampus.

In a clinical study, Bayir et al. (Bayir, 2004) examined decreases in F(2)-isoprostane (a marker of lipid peroxidation) in the cerebrospinal fluid of male and female patients with severe traumatic brain injuries treated with hypothermia. They found that F(2)-isoprostane levels were twice as high in the brain-injured males as in the females, especially on day one, when injury-induced lipid peroxidation would be highest. While the Bayir group did not measure serum progesterone levels in the females, females do have endogenously higher levels of the hormone than males, so the work indirectly supports the findings of Roof et al. (Roof et al., 1997) showing that 8-isoprostane is dramatically reduced by a single dose of exogenous progesterone given immediately after cortical contusion injury in laboratory rats. Collectively, these studies suggest that progesterone reduces lipid peroxidation and oxidative stress, most likely by decreasing the generation of free radicals and enhancing endogenous free-radical-scavenging systems.

## Inflammation

Progesterone and its active metabolites have been shown to be potent antagonists of CNS inflammation and cerebral edema after TBI. Two mechanisms, both triggered by TBI and stroke, appear to play a role: antagonism of cytokine release and inhibition of immune cell activation and migration.

**Cytokines**—Cytokines are potent activators of the inflammatory process. Administration of progestins reduces inflammation in part by modulating cytokine production and action. Contusion injuries produce a marked inflammatory reaction, with heavy gliosis seen in brain areas proximal and distal to the injury (Holmin et al., 1995). MFC contusions cause an invasion of macrophages and neutrophils into the impact area (Fulop, 1992). Both the literature and our preliminary work suggest that progesterone reduces the inflammatory immune response by blunting cytokine generation and/or action (Arvin et al., 1996; Chao, 1994; Ehring et al., 1998; Ganter et al., 1992; Hunt et al., 1997; Kelly et al., 1997; Robert and Spitzer, 1997).

To determine whether progesterone treatment could attenuate the initial surge of inflammatory cytotoxic agents after TBI, Pettus et al. extracted protein samples from the brains of frontal cortex-contused male rats 48 hours after injury and used Western blot densitometry to measure levels of the inflammatory factors C3 complement (9kDalton and 75kDalton) and NfκB p 65. These inflammatory proteins were significantly reduced within two days of starting progesterone treatment, leading to decreased edema (Pettus et al., 2005).

Both progesterone and allopregnanalone reduce injury-induced expression of inflammatory cytokines interleukin 1beta (IL-1β) and tumor necrosis factor alpha (TNFα) and the concentration of proteins regulated by the mRNA expression (Djebaili et al., 2004; Djebaili et al., 2005; He et al., 2004a; He et al., 2004b). *In vivo* studies indicate that expression of IL-1β occurs in microglia in the area of a TBI within 1 to 4 hours of injury, and in areas of reactive gliosis 24 hours to 7 days later (Pearson et al., 1999). Inhibition of IL-1β can reduce excitotoxicity and the severity of injury induced by TBI (Pearson et al., 1999). TNFα and IFNγ activate microglia *in vitro*, and administration of progesterone inhibits such microglial activity (Drew and Chavis, 2000). In addition to inhibiting the effects of TNFα on microglia,

progesterone decreases expression of TNF $\alpha$ , iNOS (protein and mRNA) and NO released by microglia (Drew and Chavis, 2000) leading to less neural degeneration and apoptosis.

These findings continue to be corroborated by others. Recently, Gibson et al. (Gibson et al., 2005) used a male mouse transient MCAO model of ischemic injury followed by IP injections of progesterone within the first 24 hours after surgery. Whether by direct or indirect mechanisms, edema was substantially reduced in the treated mice. This may have been due to the finding that the expression of the inflammatory factors  $\text{IL-1}\beta$ , TGF- $\beta$ 2 and NOS-2 were all reduced; however, the authors speculate that the hormones' effects can be mediated by either membrane-associated or intracellular progesterone receptors. However, the same group (Coughlan et al., 2005) also suggested that iNOS may be an important factor in triggering the inflammatory cytokine response, since progesterone reduces iNOS transcript in cultured astrocytes and macrophages as well as in mice subjected to MCAO.

**Inflammatory Immune Cell Activation**—Our data also suggest that cytokine reduction will decrease the expression of markers of immune cell activation and migration. Progesterone treatment following cortical contusions significantly decreases complement factors 3 and 5, macrophage-inducing factor-1, and CD4 and CD74. In addition, administering progesterone shortly after CNS injury reduces both migration and proliferation of immune cells (Nilsen and Brinton, 2002). In light of these findings, we now think that progesterone, acting through a number of different cellular mechanisms, can decrease the intensity of secondary injury, reducing the reactive glial response to injury.

### Apoptosis and DNA Repair

Progesterone can reduce apoptosis in neurons after TBI. At the genomic level, NF $\kappa$ B has recently been implicated in the initiation of inflammation and apoptosis in TBI. At the transcriptional level, progesterone reduces both the nuclear concentration of NF $\kappa$ B and expression of NF $\kappa$ B target genes.

Other pro-apoptotic enzymes, including caspase-3, BAX, and AKT, are also reduced by progesterone treatment. Contused untreated animals have significantly higher caspase-3 expression than sham surgery controls. Progesterone-treated animals had lower active caspase-3 expression, similar to that of shams ( $p < 0.05$ ). Untreated brain-injured rats had a significantly higher expression of BAX and AKT protein compared to shams and brain-injured rats treated with progesterone ( $\sim 2X$ ,  $p < 0.05$ ) (Djebaili et al., 2005).

Progesterone may also decrease apoptosis through modulation of anti-apoptotic proteins such as bcl-2 and ERK. Our previous studies have shown that progesterone up-regulates the anti-apoptotic version of bcl-2 (Djebaili et al., 2004; Djebaili et al., 2005), an anti-apoptotic gene involved in the survival of nerve growth factor dependent neurons. Bcl-2 is also thought to prevent cytochrome C release from the mitochondrial membrane, thereby preventing its binding and subsequent activation of caspase-3 (Djebaili et al., 2004; Djebaili et al., 2005)—a pathway that can lead to apoptosis and neuronal death. Untreated brain-injured animals also showed reduced expression of the anti-apoptotic protein ERK, while progesterone-treated animals exhibited upregulated expression of this protein (Ghoumari et al., 2003). In a fluid percussion injury model in male rats (Yao et al., 2005), researchers gave progesterone by injection for 1–7 days beginning within 1 hr after injury and found that the hormone substantially up-regulated the expression of the anti-apoptotic gene and protein for bcl-2, while down-regulating the gene and protein expression of both pro-apoptotic *bax* and *bad* in the damaged cortex. These authors also found that brain-injured animals receiving no treatment also have reduced expression of the anti-apoptotic protein ERK, while those treated with progesterone exhibited upregulated expression of the protective protein.

## GABAA

There is ample evidence that progesterone up-regulates GABAA, an inhibitory neurotransmitter in the CNS (Bayir, 2004; Mani, 2006; Pierson et al., 2005; Shen et al., 2005). GABA-mediated inhibition can, in turn, decrease excessive injury-induced excitotoxicity caused by the release of glutamate or other excitatory neurotransmitters (Brann et al., 2005). Increasing evidence indicates that progesterone treatment may be effective in controlling catamenial epilepsy, a condition caused by excessive levels of estrogen during certain phases of the menstrual cycle (Herzog et al., 2003, Rhodes and Frye 2005; Reddy, 2004; Rhodes and Frye, 2005).

## Myelin repair

There is growing confirmation that progesterone is implicated in increased production of myelin in both central and peripheral nervous systems. Neuronal injury reduces myelin production and leads to the loss of the sheathing oligodendrocytes. Progesterone appears to play a role in remyelinating damaged axons, at least in the spinal cord and in peripheral nerves (De Nicola et al., 2006; Gonzalez et al., 2004; Gonzalez et al., 2005; Labombarda et al., 2006).

Several recent spinal cord injury studies have shown that progesterone treatment stimulates synthesis of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) at levels of both mRNA and protein expression. Spinal cord injury can reduce BDNF levels by as much as 50%. Administration of progesterone for only three days results in a tripling of this neurotrophic factor and dramatically reduces secondary degeneration of motor neurons in the spinal cord (De Nicola et al., 2006; Gonzalez Deniselle et al., 2005; Labombarda et al., 2006).

Progesterone may also prove beneficial in chronic demyelinating conditions such as multiple sclerosis. Schumacher and colleagues (Schumacher et al., 2004) reported that progesterone treatment of symptomatic Wobbler mice with motor neuron degeneration reduced neuropathology and upregulated myelination in oligodendrocytes in the CNS. Labombarda et al. (Gonzalez et al., 2005; Labombarda et al., 2006) demonstrated that progesterone treatment increases myelin production, especially in injured animals. If these results are replicated in other species, progesterone may prove a potential treatment for certain neurodegenerative disorders such as multiple sclerosis.

## Progesterone and Medroxyprogesterone Acetate (MPA)

Synthetic and proprietary hormones such as medroxyprogesterone acetate (MPA; Provera) may have different effects from those of natural progesterone in post-injury treatment. MPA/Provera has long been widely used in hormone therapy. However, it does not mimic all the protective effects of natural progesterone, and could be a confounding variable if it were haphazardly selected for clinical testing for TBI. Our own unpublished data show that MPA can reduce cerebral edema after TBI, but unlike progesterone, MPA did not result in any behavioral recovery on the tasks we used. A few studies provide evidence that MPA acts differently in the brain than natural progesterone. Nilsen and Brinton (Nilsen and Brinton, 2002; Nilsen and Brinton, 2003) found that in hippocampal neuron cultures, estrogen and progesterone, alone or in combination, protected cells from excessive glutamate-induced calcium influx toxicity. Administration of MPA not only had no beneficial effects, it blocked estrogen-induced neuroprotection and enhanced calcium toxicity. MPA also blocked the expression of neurotrophic genes such as Bcl-2, whereas natural progesterone enhanced Bcl-2 and neuroprotection. Littleton-Kearney, Klaus and Hurn (Littleton-Kearney et al., 2005) fed conjugated estrogens in combination with MPA to female rats with ischemic injury and found

that the MPA, alone or in combination, may have counteracted the ability of the estrogen to reduce subcortical infarct volume.

Although there is continuing cause for concern in the use of synthetic progestins, one new study shows that progesterone and MPA do have some common central and peripheral effects. Following two weeks of oral administration in ovariectomized female rats, both progesterone and MPA were able to increase allopregnanolone levels in brain, but they had different effects on beta endorphin levels in the CNS (Bernardi et al., 2006). However, the issue of whether the synthetic and natural versions of the hormones can be used completely interchangeably is still not resolved. Our own unpublished research shows that after TBI, both MPA and progesterone in equivalent doses can reduce cerebral edema, but post-injury treatment with MPA, unlike progesterone, did not improve behavioral outcomes in a spatial learning task. Because of its ready availability compared to natural progesterone, it is likely that some physicians could be tempted to use MPA for “off-label” treatment but, given what we now know, caution is still required, especially in the context of brain injury or stroke. Its differential impact on outcomes compared to natural progesterone must be clarified.

## Conclusion

A large and rapidly growing body of preclinical studies have produced consistent results across species (mice, rats, cats, rabbits) and a number of injury models (TBI, stroke, spinal cord injury, demyelination). Progesterone’s most beneficial effect in TBI may be to reduce cerebral edema and thereby stem the secondary loss of vulnerable nerve cells, but the hormone exerts other beneficial effects as well. The literature indicates that progesterone is a potent anti-inflammatory and anti-apoptotic agent with some anti-oxidant properties which enable it to protect against the breakdown of cell membranes that leads to the death of neurons and glia. Progesterone’s mechanisms for neuroprotection are not yet completely understood, but it clearly does not target a single class of receptors or one cell type. Progesterone has manifold actions in the brain, so it is likely that it alters the expression of as yet unidentified genes and proteins involved in the cytotoxic and repair processes that accompany TBI and stroke.

To date, most of the pharmacological trials for TBI and stroke have failed. One reason may be that many of these drugs targeted a single aspect of the injury cascade. In contrast, progesterone, a developmental hormone that may have evolved to protect gestation of the fetus, exerts a multitude of beneficial actions, and holds promise as a safe and effective agent for a range of CNS disorders.

## References

- Alderson P, Roberts I, et al. Corticosteroids for acute traumatic brain injury. *Cochrane Database Syst Rev* 2000:CD000196. [PubMed: 10796701]
- Alderson P, Gadkary C, Signorini DF, et al. Therapeutic hypothermia for head injury. *Cochrane Database Syst Rev* 2004:CD001048. [PubMed: 15495003]
- Alderson P, Roberts I, et al. Corticosteroids for acute traumatic brain injury. *Cochrane Database Syst Rev* 2005:CD000196. [PubMed: 15674869]
- Alkayed NJ, Murphy SJ, Traystman RJ, Hurn PD, Miller VM, et al. Neuroprotective effects of female gonadal steroids in reproductively senescent female rats. *Stroke* 2000;31:161–8. [PubMed: 10625733]
- Arvin B, Neville LF, Barone FC, Feuerstein GZ, et al. The role of inflammation and cytokines in brain injury. *Neurosci Biobehav Rev* 1996;20:445–52. [PubMed: 8880734]
- Asbury ET, Fritts ME, Horton JE, Isaac WL, et al. Progesterone facilitates the acquisition of avoidance learning and protects against subcortical neuronal death following prefrontal cortex ablation in the rat. *Behav Brain Res* 1998;97:99–106. [PubMed: 9867235]

- Attella MJ, Nattinville A, Stein DG, et al. Hormonal state affects recovery from frontal cortex lesions in adult female rats. *Behav Neural Biol* 1987;48:352–67. [PubMed: 3689284]
- Auger CJ, De Vries GJ, et al. Progesterin receptor immunoreactivity within steroid-responsive vasopressin-immunoreactive cells in the male and female rat brain. *J Neuroendocrinol* 2002;14:561–7. [PubMed: 12121493]
- Baulieu EE, Robel P, et al. Neurosteroids: a new brain function? *J Steroid Biochem Mol Biol* 1990;37:395–403. [PubMed: 2257243]
- Baulieu EE, et al. Neurosteroids: of the nervous system, by the nervous system, for the nervous system. *Recent Prog Horm Res* 1997;52:1–32. [PubMed: 9238846]
- Baulieu EE, Schumacher M, et al. Neurosteroids, with special reference to the effect of progesterone on myelination in peripheral nerves. *Mult Scler* 1997;3:105–12. [PubMed: 9291163]
- Baulieu EE, et al. Neurosteroids: a novel function of the brain. *Psychoneuroendocrinology* 1998;23:963–87. [PubMed: 9924747]
- Bayir H, Marion DW, Puccio AM, Wisniewski SR, Janesko KL, Clark R, Kochanek PM, et al. Marked gender effect on lipid peroxidation after severe traumatic brain injury in adult patients. *J Neurotrauma* 2004;21:1–8. [PubMed: 14987460]
- Bernardi F, Pluchino N, Pieri M, Begliuomini S, Lenzi E, Puccetti S, Casarosa E, Luisi M, Genazzani AR, et al. Progesterone and medroxyprogesterone acetate effects on central and peripheral allopregnanolone and beta-endorphin levels. *Neuroendocrinology* 2006;83:348–59. [PubMed: 16931878]
- Betz AL, Coester HC, et al. Effect of steroids on edema and sodium uptake of the brain during focal ischemia in rats. *Stroke* 1990a;21:1199–204. [PubMed: 2389301]
- Betz AL, Coester HC, et al. Effect of steroid therapy on ischaemic brain oedema and blood to brain sodium transport. *Acta Neurochir Suppl (Wien)* 1990b;51:256–8. [PubMed: 2089910]
- Bitran D, Shiekh M, McLeod M, et al. Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain GABAA receptors. *J Neuroendocrinol* 1995;7:171–7. [PubMed: 7606242]
- Blaustein JD, et al. Progesterin receptors: neuronal integrators of hormonal and environmental stimulation. *Ann N Y Acad Sci* 2003;1007:238–50. [PubMed: 14993057]
- Brain Trauma Foundation. Guidelines for the management of severe head injury. Brain Trauma Foundation, American Association of Neurological Surgeons, Joint Section on Neurotrauma and Critical Care. *J Neurotrauma* 1996;13:641–734. [PubMed: 8941879]
- Bramlett HM, Dietrich WD, et al. Neuropathological protection after traumatic brain injury in intact female rats versus males or ovariectomized females. *J Neurotrauma* 2001;18:891–900. [PubMed: 11565601]
- Brann DW, Zamorano PL, De Sevilla L, Mahesh VB, et al. Expression of glutamate receptor subunits in the hypothalamus of the female rat during the afternoon of the proestrous luteinizing hormone surge and effects of antiprogestin treatment and aging. *Neuroendocrinology* 2005;81:120–8. [PubMed: 15961958]
- Cargill RS, Geddes DM, Malcom S, Hoffman SW, et al. Progesterone is protective at the cellular level in an in vitro model of TBI. *J Neurotrauma* 1999;16:983.
- Cervantes M, Gonzalez-Vidal MD, Ruelas R, Escobar A, Morali G, et al. Neuroprotective effects of progesterone on damage elicited by acute global cerebral ischemia in neurons of the caudate nucleus. *Arch Med Res* 2002;33:6–14. [PubMed: 11825624]
- Chao TC, Van Alten PJ, Walker RJ, et al. Steroid sex hormones and macrophage function: modulation of reactive oxygen intermediates and nitrite release. *American Journal of Reproductive Immunology* 1994;32:43–52. [PubMed: 7945814]
- Chavez-Delgado ME, Mora-Galindo J, Gomez-Pinedo U, Feria-Velasco A, Castro-Castaneda S, Lopez-Dellamary Toral FA, Luquin-De Anda S, Garcia-Segura LM, Garcia-Estrada J, et al. Facial nerve regeneration through progesterone-loaded chitosan prosthesis. A preliminary report. *J Biomed Mater Res B Appl Biomater* 2003;67:702–11. [PubMed: 14598397]
- Chen J, Chopp M, Li Y, et al. Neuroprotective effects of progesterone after transient middle cerebral artery occlusion in rat. *J Neurol Sci* 1999;171:24–30. [PubMed: 10567046]



- Clifton GL, Allen S, Barrodale P, Plenger P, Berry J, Koch S, Fletcher J, Hayes RL, Choi SC, et al. A phase II study of moderate hypothermia in severe brain injury. *J Neurotrauma* 1993;10:263–71; discussion 273. [PubMed: 8258839]
- Clifton GL, Miller ER, Choi SC, Levin HS, McCauley S, Smith KR, Muizelaar JP, Wagner FC, Marion DW, Luerssen TG, Chesnut RM, Schwartz M, et al. Lack of effect of induction of hypothermia after acute brain injury. *N Engl J Med* 2001;344:556–63. [PubMed: 11207351]
- Cochrane Collection. The Cochrane Collection Brain Injury Review. 2005
- Coughlan T, Gibson C, Murphy S, et al. Modulatory effects of progesterone on inducible nitric oxide synthase expression in vivo and in vitro. *J Neurochem* 2005;93:932–42. [PubMed: 15857396]
- Cutler SM, Pettus EH, Hoffman SW, Stein DG, et al. Tapered progesterone withdrawal enhances behavioral and molecular recovery after traumatic brain injury. *Exp Neurol* 2005;195:423–9. [PubMed: 16039652]
- Davies AR, et al. Hypothermia improves outcome from traumatic brain injury. *Crit Care Resusc* 2005;7:238–43. [PubMed: 16545052]
- De Nicola AF, Gonzalez SL, Labombarda F, Deniselle MC, Garay L, Guennoun R, Schumacher M, et al. Progesterone treatment of spinal cord injury: Effects on receptors, neurotrophins, and myelination. *J Mol Neurosci* 2006;28:3–15. [PubMed: 16632872]
- Djebaili M, Hoffman SW, Stein DG, et al. Allopregnanolone and progesterone decrease cell death and cognitive deficits after a contusion of the rat pre-frontal cortex. *Neuroscience* 2004;123:349–59. [PubMed: 14698743]
- Djebaili M, Guo Q, Pettus EH, Hoffman SW, Stein DG, et al. The neurosteroids progesterone and allopregnanolone reduce cell death, gliosis, and functional deficits after traumatic brain injury in rats. *J Neurotrauma* 2005;22:106–18. [PubMed: 15665606]
- Drew PD, Chavis JA, et al. Female sex steroids: effects upon microglial cell activation. *J Neuroimmunol* 2000;111:77–85. [PubMed: 11063824]
- Dufourny L, Skinner DC, et al. Colocalization of progesterone receptors and thyroid hormone receptors alpha in the ovine diencephalon: no effect of estradiol. *Neuroendocrinology* 2003;77:51–8. [PubMed: 12624541]
- Edwards P, Arango M, Balica L, Cottingham R, El-Sayed H, Farrell B, Fernandes J, Gogichaisvili T, Golden N, Hartzenberg B, Husain M, Ulloa MI, Jerbi Z, Khamis H, Komolafe E, Laloe V, Lomas G, Ludwig S, Mazairac G, Munoz Sanchez Mde L, Nasi L, Oildashi F, Plunkett P, Roberts I, Sandercock P, Shakur H, Soler C, Stocker R, Svoboda P, Trenkler S, Venkataramana NK, Wasserberg J, Yates D, Yutthakasemsunt S, et al. Final results of MRC CRASH, a randomised placebo-controlled trial of intravenous corticosteroid in adults with head injury-outcomes at 6 months. *Lancet* 2005;365:1957–9. [PubMed: 15936423]
- Ehring GR, Kerschbaum HH, Eder C, Neben AL, Fanger CM, Khoury RM, Negulescu PA, Cahalan MD, et al. A nongenomic mechanism for progesterone-mediated immunosuppression: inhibition of K<sup>+</sup> channels, Ca<sup>2+</sup> signaling, and gene expression in T lymphocytes. *J Exp Med* 1998;188:1593–602. [PubMed: 9802971]
- Fan L, Young PR, Barone FC, Feuerstein GZ, Smith DH, McIntosh TK, et al. Experimental brain injury induces differential expression of tumor necrosis factor-alpha mRNA in the CNS. *Brain Res Mol Brain Res* 1996;36:287–91. [PubMed: 8965649]
- Fee DB, Swartz KR, Joy KM, Roberts KN, Scheff NN, Scheff SW, et al. Effects of progesterone on experimental spinal cord injury. *Brain Res* 2007;1137:146–52. [PubMed: 17204255]
- Foroutan A, Behbahan MM, Anderson DK, et al. Effects of methylprednisolone on the GABA- and glutamate-induced currents: relevance to glucocorticoid-induced neurotoxicity and brain aging. *Steroids* 1996;61:354–66. [PubMed: 8776798]
- Fritts, ME.; Castro, EA.; Stein, DG.; Roof, RL. Progesterone, but not methylprednisolone, reduces edema in female rats after cortical contusion. Society for Neuroscience Annual Meeting; Washington, D.C.: 1996.
- Fulop, Z.; Duvdevani, R.; Hoffman, SW.; Stein, DG. 22nd Annual Meeting of the Society for Neuroscience. Vol. 18. Anaheim, CA, USA: 1992. Two step activation of the resident and invading microglial cells following brain contusion; p. 178

- Ganter S, Northoff H, Mannel D, Gebicke-Harter PJ, et al. Growth control of cultured microglia. *J Neurosci Res* 1992;33:218–30. [PubMed: 1333539]
- Garcia-Estrada J, Del Rio JA, Luquin S, Soriano E, Garcia-Segura LM, et al. Gonadal hormones down-regulate reactive gliosis and astrocyte proliferation after a penetrating brain injury. *Brain Res* 1993;628:271–8. [PubMed: 8313156]
- Garcia-Estrada J, Luquin S, Fernandez AM, Garcia-Segura LM, et al. Dehydroepiandrosterone, pregnenolone and sex steroids down-regulate reactive astroglia in the male rat brain after a penetrating brain injury. *Int J Dev Neurosci* 1999;17:145–51. [PubMed: 10221674]
- Ghoumari AM, Ibanez C, El-Etr M, Leclerc P, Eychenne B, O'Malley BW, Baulieu EE, Schumacher M, et al. Progesterone and its metabolites increase myelin basic protein expression in organotypic slice cultures of rat cerebellum. *J Neurochem* 2003;86:848–59. [PubMed: 12887683]
- Ghoumari AM, Baulieu EE, Schumacher M, et al. Progesterone increases oligodendroglial cell proliferation in rat cerebellar slice cultures. *Neuroscience* 2005;135:47–58. [PubMed: 16054770]
- Gibson CL, Murphy SP, et al. Progesterone enhances functional recovery after middle cerebral artery occlusion in male mice. *J Cereb Blood Flow Metab* 2004;24:805–13. [PubMed: 15241189]
- Gibson CL, Constantin D, Prior MJ, Bath PM, Murphy SP, et al. Progesterone suppresses the inflammatory response and nitric oxide synthase-2 expression following cerebral ischemia. *Exp Neurol* 2005;193:522–30. [PubMed: 15869954]
- Gomes JA, Stevens RD, Lewin JJ 3rd, Mirski MA, Bhardwaj A, et al. Glucocorticoid therapy in neurologic critical care. *Crit Care Med* 2005;33:1214–24. [PubMed: 15942333]
- Gonzalez Deniselle MC, Garay L, Gonzalez S, Guennoun R, Schumacher M, De Nicola AF, et al. Progesterone restores retrograde labeling of cervical motoneurons in Wobbler mouse motoneuron disease. *Exp Neurol* 2005;195:518–23. [PubMed: 16095593]
- Gonzalez SL, Labombarda F, Gonzalez Deniselle MC, Guennoun R, Schumacher M, De Nicola AF, et al. Progesterone up-regulates neuronal brain-derived neurotrophic factor expression in the injured spinal cord. *Neuroscience* 2004;125:605–14. [PubMed: 15099674]
- Gonzalez SL, Labombarda F, Deniselle MC, Mouguel A, Guennoun R, Schumacher M, De Nicola AF, et al. Progesterone neuroprotection in spinal cord trauma involves up-regulation of brain-derived neurotrophic factor in motoneurons. *J Steroid Biochem Mol Biol* 2005;94:143–9. [PubMed: 15862959]
- Goodman Y, Bruce AJ, Cheng B, Mattson MP, et al. Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons. *J Neurochem* 1996;66:1836–44. [PubMed: 8780008]
- Goss CW, Hoffman SW, Stein DG, et al. Behavioral effects and anatomic correlates after brain injury: a progesterone dose-response study. *Pharmacol Biochem Behav* 2003;76:231–42. [PubMed: 14592674]
- Gruber CJ, Huber JC. Differential effects of progestins on the brain. *Maturitas* 2003;46(Suppl 1):S71–5. [PubMed: 14670648]
- Guerra-Araiza C, Coyoy-Salgado A, Camacho-Arroyo I, et al. Sex differences in the regulation of progesterone receptor isoforms expression in the rat brain. *Brain Res Bull* 2002;59:105–9. [PubMed: 12379440]
- Gulinello M, Orman R, Smith SS, et al. Sex differences in anxiety, sensorimotor gating and expression of the alpha4 subunit of the GABAA receptor in the amygdala after progesterone withdrawal. *Eur J Neurosci* 2003;17:641–8. [PubMed: 12581182]
- Guo Q, Sayeed I, Baronne LM, Hoffman SW, Guennoun R, Stein DG, et al. Progesterone administration modulates AQP4 expression and edema after traumatic brain injury in male rats. *Exp Neurol* 2006;198:469–78. [PubMed: 16445913]
- He J, Evans CO, Hoffman SW, Oyesiku NM, Stein DG, et al. Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. *Exp Neurol* 2004a;189:404–12. [PubMed: 15380490]
- He J, Hoffman SW, Stein DG, et al. Allopregnanolone, a progesterone metabolite, enhances behavioral recovery and decreases neuronal loss after traumatic brain injury. *Restor Neurol Neurosci* 2004b; 22:19–31. [PubMed: 15096691]

- Herzog AG, Coleman AE, Jacobs AR, Klein P, Friedman MN, Drislane FW, Ransil BJ, Schomer DL, et al. Interictal EEG discharges, reproductive hormones, and menstrual disorders in epilepsy. *Ann Neurol* 2003;54:625–37. [PubMed: 14595652]
- Holmin S, Mathiesen T, Shetye J, Biberfeld P, et al. Intracerebral inflammatory response to experimental brain contusion. *Acta Neurochir (Wien)* 1995;132:110–9. [PubMed: 7754844]
- Hunt JS, Miller L, Roby KF, Huang J, Platt JS, DeBrot BL, et al. Female steroid hormones regulate production of pro-inflammatory molecules in uterine leukocytes. *J Reprod Immunol* 1997;35:87–99. [PubMed: 9421794]
- Inoue T, Akahira J, Suzuki T, Darnel AD, Kaneko C, Takahashi K, Hatori M, Shirane R, Kumabe T, Kurokawa Y, Satomi S, Sasano H, et al. Progesterone production and actions in the human central nervous system and neurogenic tumors. *J Clin Endocrinol Metab* 2002;87:5325–31. [PubMed: 12414909]
- Jiang N, Chopp M, Stein D, Feit H, et al. Progesterone is neuroprotective after transient middle cerebral artery occlusion in male rats. *Brain Res* 1996;735:101–7. [PubMed: 8905174]
- Jung-Testas I, Schumacher M, Robel P, Baulieu EE, et al. Actions of steroid hormones- and growth factors on glial cells of the central and peripheral nervous system. *J Steroid Biochem Mol Biol* 1994;48:145–54. [PubMed: 8136300]
- Jung-Testas I, Baulieu EE, et al. Steroid hormone receptors and steroid action in rat glial cells of the central and peripheral nervous system. *J Steroid Biochem Mol Biol* 1998;65:243–51. [PubMed: 9699879]
- Kelly RW, Carr GG, Riley SC, et al. The inhibition of synthesis of a beta-chemokine, monocyte chemoattractant protein-1 (MCP-1) by progesterone. *Biochem Biophys Res Commun* 1997;239:557–61. [PubMed: 9344869]
- Kleindienst A, Fazzina G, Amorini AM, Dunbar JG, Glisson R, Marmarou A. Modulation of AQP4 expression by the protein kinase C activator, phorbol myristate acetate, decreases ischemia-induced brain edema. *Acta Neurochir Suppl* 2006;96:393–7. [PubMed: 16671492]
- Koenig HL, Schumacher M, Ferzaz B, Thi AN, Ressousches A, Guennoun R, Jung-Testas I, Robel P, Akwa Y, Baulieu EE, et al. Progesterone synthesis and myelin formation by Schwann cells. *Science* 1995;268:1500–3. [PubMed: 7770777]
- Konen, JA.; Seymour, PM.; Fritts, ME.; Powell, RA.; Stein, DG. 27th Society for Neuroscience Meeting. Vol. 268. Bethesda, MD: 1997. Delayed treatment with progesterone is effective at reducing edema, delayed treatment with methylprednisolone is not.
- Kumon Y, Kim SC, Tompkins P, Stevens A, Sakaki S, Loftus CM, et al. Neuroprotective effect of postischemic administration of progesterone in spontaneously hypertensive rats with focal cerebral ischemia. *J Neurosurg* 2000;92:848–52. [PubMed: 10794300]
- Labombarda F, Gonzalez SL, Gonzalez DM, Guennoun R, Schumacher M, de Nicola AF, et al. Cellular basis for progesterone neuroprotection in the injured spinal cord. *J Neurotrauma* 2002;19:343–55. [PubMed: 11939502]
- Labombarda F, Gonzalez SL, Deniselle MC, Vinson GP, Schumacher M, De Nicola AF, Guennoun R, et al. Effects of injury and progesterone treatment on progesterone receptor and progesterone binding protein 25-Dx expression in the rat spinal cord. *J Neurochem* 2003;87:902–13. [PubMed: 14622121]
- Labombarda F, Gonzalez S, Gonzalez Deniselle MC, Garay L, Guennoun R, Schumacher M, De Nicola AF, 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;23:181–92. [PubMed: 16503802]
- Lambert JJ, Belelli D, Peden DR, Vardy AW, Peters JA, et al. Neurosteroid modulation of GABAA receptors. *Prog Neurobiol* 2003;71:67–80. [PubMed: 14611869]
- Leonelli E, Bianchi R, Cavaletti G, Caruso D, Crippa D, Garcia-Segura LM, Lauria G, Magnaghi V, Roglio I, Melcangi RC, et al. Progesterone and its derivatives are neuroprotective agents in experimental diabetic neuropathy: A multimodal analysis. *Neuroscience* 2007;144:1293–304. [PubMed: 17187935]
- Littleton-Kearney MT, Klaus JA, Hurn PD, et al. Effects of combined oral conjugated estrogens and medroxyprogesterone acetate on brain infarction size after experimental stroke in rat. *J Cereb Blood Flow Metab* 2005;25:421–6. [PubMed: 15689957]

- Lonstein JS, Blaustein JD, et al. Immunocytochemical investigation of nuclear progesterin receptor expression within dopaminergic neurones of the female rat brain. *J Neuroendocrinol* 2004;16:534–43. [PubMed: 15189328]
- Malcolm SG, Cargill RS, et al. Progesterone protects neurons from mitochondrial damage after in vitro injury. *J Neurotrauma* 2000;17:967.
- Mani SK, et al. Signaling mechanisms in progesterone-neurotransmitter interactions. *Neuroscience* 2006;138:773–81. [PubMed: 16310962]
- Marmarou A. The pathophysiology of brain edema and elevated intracranial pressure. *Cleve Clin J Med* 2004;71(Suppl 1):S6–8. [PubMed: 14964471]
- Marmarou A, Signoretti S, Aygok G, Fatouros P, Portella G. Traumatic brain edema in diffuse and focal injury: cellular or vasogenic? *Acta Neurochir Suppl* 2006a;96:24–9. [PubMed: 16671417]
- Marmarou A, Signoretti S, Fatouros PP, Portella G, Aygok GA, Bullock MR, et al. Predominance of cellular edema in traumatic brain swelling in patients with severe head injuries. *J Neurosurg* 2006b;104:720–30. [PubMed: 16703876]
- Maurice T, Phan VL, Urani A, Guillemain I, et al. Differential involvement of the sigma(1) (sigma(1)) receptor in the anti-amnesic effect of neuroactive steroids, as demonstrated using an in vivo antisense strategy in the mouse. *Br J Pharmacol* 2001;134:1731–41. [PubMed: 11739250]
- Meffre D, Delespierre B, Guezou M, Leclerc P, Vinson GP, Schumacher M, Stein DG, Guennoun R, 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;93:1314–26. [PubMed: 15934950]
- Monnet FP, Maurice T, et al. The sigma1 protein as a target for the non-genomic effects of neuro(steroid) steroids: molecular, physiological, and behavioral aspects. *J Pharmacol Sci* 2006;100:93–118. [PubMed: 16474209]
- Morali G, Letechipia-Vallejo G, Lopez-Loeza E, Montes P, Hernandez-Morales L, Cervantes M, et al. Post-ischemic administration of progesterone in rats exerts neuroprotective effects on the hippocampus. *Neurosci Lett* 2005;382:286–90. [PubMed: 15885907]
- Murphy SJ, Traystman RJ, Hurn PD, Duckles SP, et al. Progesterone exacerbates striatal stroke injury in progesterone-deficient female animals. *Stroke* 2000;31:1173–8. [PubMed: 10797182]
- Murphy SJ, Littleton-Kearney MT, Hurn PD, et al. Progesterone administration during reperfusion, but not preischemia alone, reduces injury in ovariectomized rats. *J Cereb Blood Flow Metab* 2002;22:1181–8. [PubMed: 12368656]
- Nilsen J, Brinton RD, et al. Impact of progestins on estradiol potentiation of the glutamate calcium response. *Neuroreport* 2002;13:825–30. [PubMed: 11997695]
- Nilsen J, Brinton RD, et al. Divergent impact of progesterone and medroxyprogesterone acetate (Provera) on nuclear mitogen-activated protein kinase signaling. *Proc Natl Acad Sci U S A* 2003;100:10506–11. [PubMed: 12925744]
- O'Connor CA, Cernak I, Vink R, et al. Both estrogen and progesterone attenuate edema formation following diffuse traumatic brain injury in rats. *Brain Res* 2005;1062:171–4. [PubMed: 16256079]
- O'Connor CA, Cernak I, Johnson F, Vink R, et al. Effects of progesterone on neurologic and morphologic outcome following diffuse traumatic brain injury in rats. *Exp Neurol*. 2007In Press, Corrected Proof, Available online 12 February 2007
- Ockene JK, Barad DH, Cochrane BB, Larson JC, Gass M, Wassertheil-Smoller S, Manson JE, Barnabei VM, Lane DS, Brzyski RG, Rosal MC, Wylie-Rosett J, Hays J, et al. Symptom experience after discontinuing use of estrogen plus progestin. *Jama* 2005;294:183–93. [PubMed: 16014592]
- Ozawa H, et al. Steroid Hormones, their receptors and neuroendocrine system. *J Nippon Med Sch* 2005;72:316–25. [PubMed: 16415511]
- Papadopoulos MC, Krishna S, Verkman AS, et al. Aquaporin water channels and brain edema. *Mt Sinai J Med* 2002;69:242–8. [PubMed: 12357265]
- Pearson VL, Rothwell NJ, Toulmond S, et al. Excitotoxic brain damage in the rat induces interleukin-1beta protein in microglia and astrocytes: correlation with the progression of cell death. *Glia* 1999;25:311–23. [PubMed: 10028914]

- Pemberton PL, Dinsmore J, et al. The use of hypothermia as a method of neuroprotection during neurosurgical procedures and after traumatic brain injury: a survey of clinical practice in Great Britain and Ireland. *Anaesthesia* 2003;58:370–3. [PubMed: 12688273]
- Pettus EH, Wright DW, Stein DG, Hoffman SW, et al. Progesterone treatment inhibits the inflammatory agents that accompany traumatic brain injury. *Brain Res* 2005;1049:112–9. [PubMed: 15932748]
- Pierson RC, Lyons AM, Greenfield LJ Jr. Gonadal steroids regulate GABAA receptor subunit mRNA expression in NT2-N neurons. *Brain Res Mol Brain Res* 2005;138:105–15. [PubMed: 16000229]
- Reddy DS, et al. Role of neurosteroids in catamenial epilepsy. *Epilepsy Res* 2004;62:99–118. [PubMed: 15579299]
- Reddy DS, O'Malley BW, Rogawski MA, et al. Anxiolytic activity of progesterone in progesterone receptor knockout mice. *Neuropharmacology* 2005;48:14–24. [PubMed: 15617723]
- Rhodes ME, Frye CA, et al. Actions at GABA(A) receptors in the hippocampus may mediate some antiseizure effects of progestins. *Epilepsy Behav* 2005;6:320–7. [PubMed: 15820338]
- Robert R, Spitzer JA, et al. Effects of female hormones (17beta-estradiol and progesterone) on nitric oxide production by alveolar macrophages in rats. *Nitric Oxide* 1997;1:453–62. [PubMed: 9466950]
- Roberts I, Schierhout G, Alderson P, et al. Absence of evidence for the effectiveness of five interventions routinely used in the intensive care management of severe head injury: a systematic review [see comments]. *J Neurol Neurosurg Psychiatry* 1998;65:729–33. [PubMed: 9810947]
- Roberts I, Yates D, Sandercock P, Farrell B, Wasserberg J, Lomas G, Cottingham R, Svoboda P, Brayley N, Mazairac G, Laloe V, Munoz-Sanchez A, Arango M, Hartzenberg B, Khamis H, Yutthakasemsunt S, Komolafe E, Oildashi F, Yadav Y, Murillo-Cabezas F, Shakur H, Edwards P, et al. Effect of intravenous corticosteroids on death within 14 days in 10008 adults with clinically significant head injury (MRC CRASH trial): randomised placebo-controlled trial. *Lancet* 2004;364:1321–8. [PubMed: 15474134]
- Robertson CL, Puskar A, Hoffman GE, Murphy AZ, Saraswati M, Fiskum G, et al. Physiologic progesterone reduces mitochondrial dysfunction and hippocampal cell loss after traumatic brain injury in female rats. *Exp Neurol* 2006;197:235–43. [PubMed: 16259981]
- Roof RL, Duvdevani R, Stein DG, et al. Gender influences outcome of brain injury: progesterone plays a protective role. *Brain Res* 1993a;607:333–6. [PubMed: 8481809]
- Roof RL, Zhang Q, Glasier MM, Stein DG, et al. Gender-specific impairment on Morris water maze task after entorhinal cortex lesion. *Behav Brain Res* 1993b;57:47–51. [PubMed: 8292254]
- Roof RL, Duvdevani R, Braswell L, Stein DG, et al. Progesterone facilitates cognitive recovery and reduces secondary neuronal loss caused by cortical contusion injury in male rats. *Exp Neurol* 1994;129:64–9. [PubMed: 7925843]
- Roof RL, Duvdevani R, Heyburn JW, Stein DG, et al. Progesterone rapidly decreases brain edema: treatment delayed up to 24 hours is still effective. *Exp Neurol* 1996;138:246–51. [PubMed: 8620923]
- Roof RL, Hoffman SW, Stein DG, et al. Progesterone protects against lipid peroxidation following traumatic brain injury in rats. *Mol Chem Neuropathol* 1997;31:1–11. [PubMed: 9271001]
- Roof, RL.; Duvdevani, R.; Heyburn, JW.; Stein, DG. Twenty-first Annual Meeting of the Society for Neuroscience. Miami Beach FL: 1994. Progesterone reduces BBB damage following bilateral, medial frontal contusion; p. 191
- Roof RL, Fritts ME, et al. Progesterone metabolites may mediate its neuroprotective effects after traumatic brain injury. *Neurotrauma* 1997;14:760.
- Roof RL, Stein DG, et al. Progesterone treatment attenuates brain edema following contusion injury in male and female rats. *Restor Neurol Neurosci* 1992;4:425–427.
- Sakamoto H, Shikimi H, Ukena K, Tsutsui K, et al. Neonatal expression of progesterone receptor isoforms in the cerebellar Purkinje cell in rats. *Neurosci Lett* 2003;343:163–6. [PubMed: 12770688]
- Sayeed I, Guo Q, Hoffman SW, Stein DG, et al. Allopregnanolone, a progesterone metabolite, is more effective than progesterone in reducing cortical infarct volume after transient middle cerebral artery occlusion. *Ann Emerg Med* 2006;47:381–9. [PubMed: 16546625]
- Sayeed I, Wali B Stein DG, et al. Progesterone inhibits ischemic brain injury in a rat model of permanent middle cerebral artery occlusion. *Restor Neurol Neurosci*. 2007in press



- Schumacher M, Baulieu EE, et al. Neurosteroids: synthesis and functions in the central and peripheral nervous systems. *Ciba Found Symp* 1995;191:90–106; discussion 106–12. [PubMed: 8582208]
- Schumacher M, Robel P, Baulieu EE, et al. Development and regeneration of the nervous system: a role for neurosteroids. *Dev Neurosci* 1996;18:6–21. [PubMed: 8840083]
- Schumacher M, Guennoun R, Robert F, Carelli C, Gago N, Ghoumari A, Gonzalez Deniselle MC, Gonzalez SL, Ibanez C, Labombarda F, Coirini H, Baulieu EE, De Nicola AF, et al. Local synthesis and dual actions of progesterone in the nervous system: neuroprotection and myelination. *Growth Horm IGF Res* 2004;14(Suppl A):S18–33. [PubMed: 15135772]
- Seppelt I, et al. Hypothermia does not improve outcome from traumatic brain injury. *Crit Care Resusc* 2005;7:233–7. [PubMed: 16545051]
- Shear DA, Galani R, Hoffman SW, Stein DG, et al. Progesterone protects against necrotic damage and behavioral abnormalities caused by traumatic brain injury. *Exp Neurol* 2002;178:59–67. [PubMed: 12460608]
- Shen H, Gong QH, Yuan M, Smith SS, et al. Short-term steroid treatment increases delta GABAA receptor subunit expression in rat CA1 hippocampus: pharmacological and behavioral effects. *Neuropharmacology* 2005;49:573–86. [PubMed: 15950994]
- Shimamura K, Sugino N, Yoshida Y, Nakamura Y, Ogino K, Kato H, et al. Changes in lipid peroxide and antioxidant enzyme activities in corpora lutea during pseudopregnancy in rats. *J Reprod Fertil* 1995;105:253–7. [PubMed: 8568768]
- Smith SS, Gong QH, et al. Neurosteroid administration and withdrawal alter GABAA receptor kinetics in CA1 hippocampus of female rats. *J Physiol* 2005;564:421–36. [PubMed: 15705652]
- Soares HD, Hicks RR, Smith D, McIntosh TK, et al. Inflammatory leukocytic recruitment and diffuse neuronal degeneration are separate pathological processes resulting from traumatic brain injury. *J Neurosci* 1995a;15:8223–33. [PubMed: 8613756]
- Soares HD, Sinson GP, McIntosh TK, et al. Fetal hippocampal transplants attenuate CA3 pyramidal cell death resulting from fluid percussion brain injury in the rat. *J Neurotrauma* 1995b;12:1059–67. [PubMed: 8742134]
- Stein DG, et al. Brain damage, sex hormones and recovery: a new role for progesterone and estrogen? *Trends Neurosci* 2001;24:386–91. [PubMed: 11410269]
- Stein, DG. The Case for Progesterone. In: Singh, JW SM., editor. *The Future of Estrogen and Hormone Therapy in Postmenopausal Women: What Basic Science and Clinical Studies Teach Us*. Vol. 1052. *Annals of the New York Academy of Sciences*; New York: 2005. p. 152-169.
- Stoffel EC, Craft RM, et al. Ovarian hormone withdrawal-induced “depression” in female rats. *Physiol Behav* 2004;83:505–13. [PubMed: 15581673]
- Subramanian M, Pusphendran CK, Tarachand U, Devasagayam TP, et al. Gestation confers temporary resistance to peroxidation in the maternal rat brain. *Neurosci Lett* 1993;155:151–4. [PubMed: 8377944]
- Temkin NR, Anderson GD, Winn HR, Ellenbogen RG, Britz GW, Schuster J, Lucas T, Newell DW, Mansfield PN, Machamer JE, Barber J, Dikmen SS, et al. Magnesium sulfate for neuroprotection after traumatic brain injury: a randomised controlled trial. *Lancet Neurol* 2007;6:29–38. [PubMed: 17166799]
- Thomas AJ, Nockels RP, Pan HQ, Shaffrey CI, Chopp M, et al. Progesterone is neuroprotective after acute experimental spinal cord trauma in rats. *Spine* 1999;24:2134–8. [PubMed: 10543012]
- Toung TJ, Chen TY, Littleton-Kearney MT, Hurn PD, Murphy SJ, et al. Effects of combined estrogen and progesterone on brain infarction in reproductively senescent female rats. *J Cereb Blood Flow Metab* 2004;24:1160–6. [PubMed: 15529016]
- Uhler TA, Frim DM, Pakzaban P, Isacson O, et al. The effects of megadose methylprednisolone and U-78517F on toxicity mediated by glutamate receptors in the rat neostriatum. *Neurosurgery* 1994;34:122–7; discussion 127–8. [PubMed: 7907169]
- Vanlandingham JW, Cutler SM, Virmani S, Hoffman SW, Covey DF, Krishnan K, Hammes SR, Jamnongjit M, Stein DG, et al. The enantiomer of progesterone acts as a molecular neuroprotectant after traumatic brain injury. *Neuropharmacology* 2006;51:1078–1085. [PubMed: 16926035]

- Vedder H, Anthes N, Stumm G, Wurz C, Behl C, Krieg JC, et al. Estrogen hormones reduce lipid peroxidation in cells and tissues of the central nervous system. *J Neurochem* 1999;72:2531–8. [PubMed: 10349864]
- Vink R, Van Den Heuvel C, et al. Recent advances in the development of multifactorial therapies for the treatment of traumatic brain injury. *Expert Opin Investig Drugs* 2004;13:1263–74.
- Wagner AK, Willard LA, Kline AE, Wenger MK, Bolinger BD, Ren D, Zafonte RD, Dixon CE, et al. Evaluation of estrous cycle stage and gender on behavioral outcome after experimental traumatic brain injury. *Brain Res* 2004;998:113–21. [PubMed: 14725974]
- Watson NF, Barber JK, Doherty MJ, Miller JW, Temkin NR, et al. Does glucocorticoid administration prevent late seizures after head injury? *Epilepsia* 2004;45:690–4. [PubMed: 15144437]
- Wright DW, Bauer ME, Hoffman SW, Stein DG, et al. Serum progesterone levels correlate with decreased cerebral edema after traumatic brain injury in male rats. *J Neurotrauma* 2001;18:901–9. [PubMed: 11565602]
- Wright DW, Kellermann AL, Hertzberg VS, Clark PL, Frankel M, Goldstein FC, Salomone JP, Dent LL, Harris OA, Ander DS, Lowery DW, Patel MM, Denson DD, Gordon AB, Wald MM, Gupta S, Hoffman SW, Stein DG, et al. ProTECT: A Randomized Clinical Trial of Progesterone for Acute Traumatic Brain Injury. *Ann Emerg Med* 2007;49:391–402. [PubMed: 17011666]
- Yao XL, Liu J, Lee E, Ling GS, McCabe JT, et al. Progesterone differentially regulates pro- and anti-apoptotic gene expression in cerebral cortex following traumatic brain injury in rats. *J Neurotrauma* 2005;22:656–68. [PubMed: 15941375]