Hypothesis

Is Progesterone a Candidate Neuroprotective Factor for Treatment following Ischemic Stroke?

The Neuroscientist
Volume 15 Number 4
August 2009 324-332

10.1177/1073858409333069
http://nro.sagepub.com

Claire L Gibson, Ben Coomber, and James Rathbone

Gender differences in stroke outcome have implicated steroid hormones as potential neuroprotective candidates. However, no clinical trials examining hormone replacement therapy on outcome following ischemic stroke have investigated the effect of progesterone-only treatment. In this review the authors examine the experimental evidence for the neuroprotective potential of progesterone and give an insight into potential mechanisms of action following ischemic stroke. To date, 17 experimental studies have investigated the neuroprotective potential of progesterone for ischemic stroke in terms of ability to both reduce cell loss and increase functional outcome. Of these 17 published studies the majority reported a beneficial effect with three studies reporting a nil effect and only one study reporting a negative effect. However, there are important issues that the authors address in this review in terms of the methodological quality of studies in relation to the STAIR recommendations. In terms of the proposed mechanisms of progesterone neuroprotection we show that progesterone is versatile and acts at multiple targets to facilitate neuronal survival and minimize cell damage and loss. A large amount of experimental evidence indicates that progesterone is a neuroprotective candidate for ischemic stroke; however, to progress to clinical trial a number of key experimental studies remain outstanding.

Keywords: progesterone; focal ischemia; neuroprotection; steroids; stroke

Stroke is the leading cause of neurological disability and a major cause of death in the western world (Lo and others 2003). More than 80% of strokes are ischemic, resulting from vascular occlusion and typically affecting the middle cerebral artery. Despite advances in the understanding of the pathophysiological events that underlie cerebral ischemia, successful therapies to treat stroke are limited (Donnan and others 2008) both in their utility (e.g., thrombolysis) and efficacy (e.g., aspirin). Ischemic stroke triggers a complex cascade of events resulting in both cell death and a loss of function, and current treatments do little to alleviate such dysfunction. There is a need for experimental studies to identify potential neuroprotective candidates and to determine whether clinical investigation is warranted.

On the basis of a large wealth of experimental evidence supporting the neuroprotective role of progesterone following traumatic brain injury (TBI; Stein and others 2008; Stein 2005), the first randomized clinical trial of progesterone for acute TBI (ProTECT) was successfully completed in 2007. This trial found that the use of progesterone following TBI was well tolerated in terms of safety and resulted in a lower 30-day mortality rate compared with the placebo group (Wright and others 2007). A subsequent clinical trial has shown improved neurological outcome in progesterone-treated TBI patients (Xiao and others 2008). Here we review the experimental evidence of progesterone and ischemic stroke to establish whether a clinical trial for progesterone-only treatment of ischemic stroke is warranted.

Steroid Hormones and Ischemic Stroke

In terms of ischemic stroke, there is a clear gender difference both in vulnerability to stroke and, indeed, outcome (Murphy and others 2004). It is clear that women have a lower risk of stroke, relative to men of the same age, prior to the onset of menopause (Sacco and others 1997; Turtzo and McCullough 2008). However, with declining circulating levels of estrogen and progesterone after menopause, the incidence of stroke in women rapidly increases (Prencipe and others 1997; Wenger and others 1993). As a result of this gender difference in stroke risk and outcome following stroke, clinical trials have examined the effect of hormone replacement therapy (HRT) following stroke.

The Heart and Estrogen-Progestin Replacement Study (HERS) and its follow-up cohort (HERS II) were the first randomized, blinded clinical trials that examined the effects of combined HRT on the progression of coronary heart disease and included stroke (and transient ischemic attacks) as secondary endpoints. HERS found that for the primary endpoint of nonfatal

From the School of Psychology, University of Leicester, Leicester, United Kingdom.

Research in the laboratory is currently funded by Research Into Ageing (317 to CLG).

Address correspondence to: Dr. Claire L Gibson, School of Psychology, University of Leicester, Henry Wellcome Building, Lancaster Road, Leicester, LE1 9HN, UK; e-mail: cg95@le.ac.uk.

or coronary heart disease there was no net reduction for those receiving HRT (Hulley and others 1998), and HERS II found that the risk of nonfatal stroke, fatal stroke, and transient ischemic attacks was not associated with hormone therapy (Simon and others 2001). In contrast, the Women's Estrogen for Stroke Trial (WEST) was the first randomized trial to examine stroke occurrence as the primary endpoint following estrogen-only HRT. The WEST trial concluded that there was no reduction in morbidity following estrogen therapy, and, in fact, following estrogen therapy neurological and functional outcomes were slightly worse following ischemic stroke (Viscoli and others 2001).

The most recent clinical trial examining the effects of HRT on stroke occurrence in postmenopausal women is the Women's Health Initiative (WHI) trial. The WHI trial was designed to test the hypothesis that estrogens (alone or in combination with progesterone) protect against cardiovascular disease and included stroke as a secondary outcome (Wassertheil-Smoller and others 2003). However, the study, which was comprised of two independent arms (i.e., estrogen-only and estrogen + progestin), was halted prematurely due to increasing hazards in both treatment groups. Initially the estrogen + progestin arm was stopped early due to an increased risk of invasive breast cancer although subsequent analyses also revealed an increased risk of stroke in this combination group. Subsequently, the estrogen-only arm of the trial was also halted prematurely due to a small increase in stroke risk. The similarity in the results between the two independent arms of the WHI trial, that is, estrogen-only and estrogen + progestin, substantially strengthened the evidence that the risk of ischemic stroke increased following HRT. Furthermore, this trial implicated estrogen, present in both arms of the trial, as the mediator of this increased risk of ischemic stroke. In support of this, there was no heterogeneity (or even trend) to dual therapy being worse than estrogen-only HRT, and so it is unlikely that long-term progesterone treatment contributed to the negative findings. In addition, such clinical trials tend to administer synthetic progestins and equine-derived estrogens rather than administering the natural hormones and it is likely that such synthetic progestins (and estrogens) will have different biological effects than their natural counterparts (Turgeon and others 2004).

Gender Differences in Cerebral Ischemic Animal Models

Experimental studies have shown a neuroprotective effect of female gender following global or focal cerebral ischemia (Alkayed and others 1998; Hurn and Macrae 2000; Hurn and others 2002; Wise and others 2001) even in the presence of specific stroke risk factors such as diabetes (Toung and others 2000) and hypertension (Alkayed and others 1998). This neuroprotective effect of female gender is abolished by ovariectomy and reproductive senescence (Alkayed and others 1998, 2000), which both result in a decline in the circulating levels of the sex steroid hormones estrogen and progesterone. However, ovariectomy and reproductive senescence do not reveal the specific contribution of progesterone to this neuroprotection. Historically, experimental work has focused on investigating the neuroprotective potential of estrogens, and only recently has progesterone been considered as a candidate. Although the evidence for estrogens as a neuroprotective agent following experimental stroke is strong (for review, see Gibson and others 2006), under certain conditions, whether related to strain, dose, or severity of ischemia, detrimental influences of estrogens have also been uncovered (Bingham and others 2005; Carswell and others 2004; Macrae and Carswell 2006).

Progesterone Is Neuroprotective in Cerebral Ischemic Animal Models

Recently, the neuroprotective potential of progesterone following cerebral ischemia has been demonstrated. Following an extensive literature search using PubMed, Embase, Web of Science, and reference lists of all identified publications and review articles, we identified 17 published studies (see Table 1) that have investigated the effect of administering progesterone either prior to or immediately following cerebral ischemia. Of these, 14 studies reported positive findings including a reduction in cell death and improvements in functional outcomes. Three studies reported a nil effect of progesterone treatment (Cai and others 2008; Choi and others 2004; Toung and others 2004), and only one study reported a detrimental effect of progesterone treatment (Murphy and others 2000). One study reported both a nil effect and beneficial effect of progesterone treatment depending on the timing of administration of the first dose of progesterone (Cai and others 2008), in that progesterone was neuroprotective when administered 48 h or 1 h prior to ischemia but failed to have an effect when administered 96 h prior to the onset of ischemia.

The studies that reported a nil effect of progesterone treatment all administered progesterone for varying times prior to the onset of ischemia, whereas the majority of studies that demonstrated a beneficial effect of progesterone administered progesterone immediately following the onset of ischemia. Those studies that administered progesterone prior to ischemia tended to abruptly halt progesterone administration at the onset of ischemia. Tapering of progesterone administration, rather than abruptly halting treatment, has been shown

Table 1. All Published Experimental Studies to Date That Investigated Whether Progesterone Was Beneficial, Detrimental, or Had no Effect following Ischemic Stroke

First Author	Year	First Dose Timing[a]	Sex/Hormonal Status	Species	Ischemia	Outcome(s)	Study Quality[b]	Beneficial (+), Detrimental (–), or No Effect (n)
Alkayed	2000	–7 d	RSF	WR	TF	Cell death	2	+
Murphy	2002	–7 d	OF	WR	TF	Cell death	4	+
Cervantes	2002	–7 d	OF	C	G	Cell death	5	+
Gonzalez-Vidal	1998	–7 d	OF	C	G	Cell death	5	+
Cai	2008	–96 h	M	SDR	TF	Cell death, behavior	4	n, n
		–48 h				Cell death, behavior	4	+, +
		–1 h				Cell death, behavior	4	+, +
Choi	2004	–24 h	M	SDR	TF	Cell death	2	n
Jiang	1996	–0.5 h, +2 h	M	WR	TF	Cell death, ND	5	+, +
Murphy	2000	–0.5 h	OF	WR	TF	Cell death	4	–
Toung	2004	–0.5 h	RSF	WR	TF	Cell death	3	n
Aggarwal	2008	–0.5 h	M	SAM	TF	Cell death	4	+
Morali	2005	+0.33 h	M	SDR	TG	Cell death	3	+
Gibson	2004	+1 h	M	CM	TF	Cell death, behavior	6	+, +
Gibson	2005	+1 h	M	CM	PF	Cell death	4	+
Sayeed	2007	+1 h	M	CDR	PF	Cell death, behavior	4	+, +
Chen	1999	+2 h	M	WR	TF	Cell death, ND	5	+, +
Kumon	2000	+2 h	M	SHRSP	TF	Cell death, ND	7	+, +
Sayeed	2006	+2 h	M	SDR	TF	Cell death	4	+

WR = Wistar rats; C = cats; SDR = Sprague-Dawley rats; SAM = Swiss Albino mice; CM = C57 mice; SHRSP = stroke-prone spontaneously hypertensive rats; RSF = reproductively senescent females; M = males; OF = ovariectomized females; PF = permanent focal; TF = transient focal; G = global; ND = neurological deficit.

[a]First dose timing in relation to onset of ischemia.

[b]Study quality according to STAIR recommendations.

to have a greater beneficial effect following TBI (Cutler and others 2006a). In addition, continued administration of progesterone, rather than bolus injections, has been demonstrated to produce a greater beneficial effect in terms of functional recovery following TBI, which may be of more clinical relevance (Cutler and others 2006b).

In terms of detrimental effects of progesterone administration, one study reported that progesterone exacerbated the lesion volume produced following cerebral ischemia when given to ovariectomized females (Murphy and others 2000). However, that was only true when progesterone was administered prior to cerebral ischemia, and those same authors went on to demonstrate that ovariectomized females that receive progesterone both before and after cerebral ischemia experienced reduced lesion volume (Murphy and others 2002). In males, progesterone has been found to be neuroprotective whether administered before or after ischemia.

Although the longest time interval between onset of ischemia and initiation of treatment that has been currently investigated is 2 h, further studies are required that extend this time point of administration. However, one published study has demonstrated that progesterone is still effective at reducing edema volume when administered at 24 h following TBI (Roof and others 1996). Moreover, the neuroprotective effects of progesterone have been demonstrated following both focal and global ischemia (Aggarwal and others 2008; Cervantes and others 2002; González-Vidal and others 1998; Moralí and others 2005). In terms of focal cerebral ischemia, neuroprotective effects were observed following the induction of both transient (Gibson and Murphy 2004) and permanent (Gibson and others 2005) cerebral ischemia. All of these studies have been conducted in rodents, and recent recommendations have highlighted the importance of evaluating putative neuroprotective treatments in primate models of cerebral ischemia with assessment of long-term function, which has not occurred for progesterone (STAIR 1999).

The majority of experimental studies tend to use lesion volume as the main outcome following cerebral ischemia. However, lesion volume may be of limited value when interpreting whether a treatment is beneficial. Functional outcome, in combination with histopathological outcome, is as important, in terms of assessing benefit (STAIR 1999), because infarct size may (Rogers and others 1997) or may not (Hattori and others 2000; Reglodi and others 2003) correlate with neurological impairment. Interestingly, one study found that allopregnanolone, a progesterone metabolite, was more effective than progesterone at reducing cortical infarct following cerebral ischemia (Sayeed and others 2006) and such metabolites warrant further investigation.

The methodological quality of each study (Table 1) was assessed using an eight-point STAIR rating as previously described (Macleod and others 2005; Gibson and others 2006, 2008) whereby one point is given for written evidence of each of the following criteria: presence of randomization, monitoring of physiological parameters, assessment of dose-response relationship, assessment of optimal time window, masked outcome measurement, assessment of outcome at days 1 to 3, assessment of outcome at days 1 to 30, combined measurement of lesion volume and functional outcome. The median quality rating of these 17 articles (Table 1) was 4 of 8 (range 2–7). This method of assigning a quality score is based upon recommendations by the STAIR (1999) for preclinical investigation of new neuroprotective agents. The publication of these recommendations for preclinical neuroprotective drug development was organized by an expert panel of stroke researchers to address why so many clinical trials of neuroprotective drugs for acute ischemic stroke have failed. As this review shows, although the evidence for progesterone neuroprotection before or after ischemia is strong, a large portion of these studies do not score high in terms of quality relevant to the recommendations proposed by STAIR.

The preclinical STAIR recommendations for the evaluation of neuroprotective agents emphasize the importance of rigorously testing such agents in preclinical stroke models designed to investigate dose ranges, the time window of treatment effects, histological and functional outcomes, sustained treatment effects and safety, before progressing to phase III efficacy trials. They also recommended that following demonstration of a biological effect in rodents such agents should be investigated in larger species/primate models. These recommendations were extended in 2003 to include the extent of histological protection, subcortical protection, white matter protection, the length of treatment, and efficacy as monotherapy (Green and others 2003). However, not all researchers would agree that it is necessary to meet all of the STAIR guidelines prior to advancing clinical trials and adherence to these guidelines has yet to prove predictive of translational success (Ginsberg 2009).

Proposed Mechanisms of Progesterone-Mediated Neuroprotection

Progesterone acts as a multifaceted messenger within the CNS (Singh 2006), capable of modulating mood, cognition, neurogenesis, axon myelination, inflammation, and recovery from various types of injury. These effects are mediated via a range of progesterone receptors (PR) including the classical nuclear PRA and PRB types, a seven-transmembrane domain receptor (7TMPRβ), and a membrane progesterone binding protein (25-Dx). Progesterone receptors are expressed broadly throughout the brain and by all types of neurons (reviewed in Brinton and others 2008). The specific signaling mechanisms by which progesterone exerts neuroprotective effects following cerebral ischemia are currently unclear, but it does appear that progesterone is versatile, acting at multiple targets to facilitate neuronal survival and to minimize cell damage and loss. As shown in Figure 1 this review focuses on what we consider to be the main putative mechanisms of progesterone-mediated neuroprotection following cerebral ischemia.

Inflammation and Cerebral Edema

Progesterone-mediated neuroprotection occurs, to an extent, through suppression of the injury-induced inflammatory response. A reduction in the expression of inflammatory mediators including interleukin-1β (IL-1β), transforming growth factor-β_2 (TGFβ_2), and nitric oxide synthase-2 (NOS-2), and a simultaneous reduction in edema volume has been demonstrated in experimental stroke studies using either progesterone (Gibson and others 2005) or its metabolite, allopregnanolone (He and others 2004). In addition, in vitro and in vivo studies reveal that progesterone directly modulates NOS-2 expression suggesting NOS-2 is involved in mediating the neuroprotective effects of progesterone following stroke (Coughlan and others 2005).

Ischemic stroke triggers an inflammatory response, which results in the formation of both cytotoxic and vasogenic edema. Cerebral edema following ischemic stroke accounts for a substantial portion of stroke-associated morbidity and mortality (Gebel and others 2002). Experimental studies have shown that progesterone administration does reduce edema formation following cerebral ischemia (Gibson and others 2005). The modulation of water removal by progesterone treatment is not fully understood, but there are some interesting clues as to potential targets, namely the membrane progesterone binding protein 25-Dx and aquaporins (APQ).

The membrane progesterone binding protein, 25-Dx, commonly expressed alongside progesterone receptors, may play a role in progesterone-mediated osmoregulation after brain injury (for review, see Guennoun and others 2008). There is an abundance of 25-Dx in brain regions proximal to CSF, including regions of the hypothalamus involved in osmoregulation and an injury-induced increase in 25-Dx expression colocalized specifically to reactive astrocytes, has been demonstrated following TBI (Meffre and others 2005). In terms of osmoregulation 25-Dx may act directly or via vasopressin (see Fig. 1), which regulates water balance and stabilizes fluid osmolarity at the

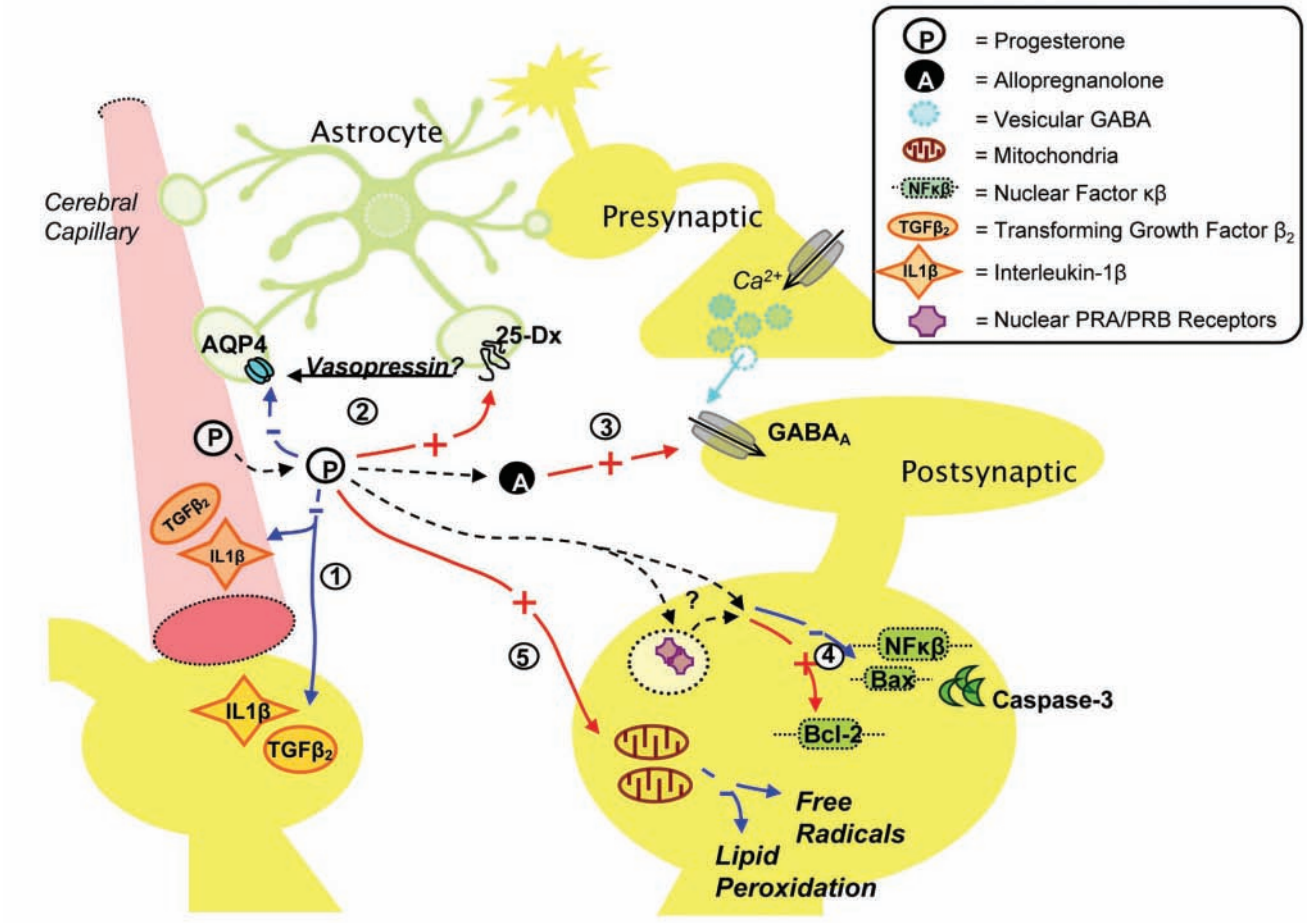

Figure 1. Proposed mechanisms of progesterone-mediated neuroprotection following ischemic stroke. Progesterone readily crosses the blood-brain barrier and it acts via the classical progesterone receptor or via nongenomic sites of action (e.g., the membrane progesterone binding protein, 25-Dx, or the $GABA_A$ receptor) to regulate cellular events important for neuroprotection such as 1) inflammation via suppressing the expression of inflammatory mediators, such as interleukin-1β and transforming growth factor-β_2; 2) edema—via 25-Dx or aquaporins directly or via vasopressin induction following binding to 25-Dx; 3) excitotoxicity—in particular, its metabolite allopregnanolone interacts with $GABA_A$ receptors to enhance $GABA_A$ function thus suppressing excitatory neurotransmission and consequently reducing excitotoxicity; 4) apoptosis and DNA repair via inhibition of the expression of transcription factors (e.g., NFKβ) and pro-apoptotic genes (e.g., caspase-3, Bax) and/or modulation of the expression of anti-apoptotic genes (e.g., Bcl-2); and 5) lipid peroxidation and oxidative stress.

cellular level of the brain parenchyma and regulates water permeability in subpial astrocytes. In the hypothalamus 25-Dx is coexpressed with vasopressin in neuronal populations that lack the nuclear PR suggesting that progesterone-mediated modulation of vasopressin may involve 25-Dx. Vasopressin may then act via the modulation of AQP, a class of water channel membrane proteins that modulate cerebral edema (Niermann and others 2001).

The primary water channel in the brain is AQP4, which is located predominantly in the endfeet of astrocytes (Rash and others 1998). AQP4 acts as an osmosensor, controls water drainage in the ventricles of the brain, and thus represents an attractive target for neuroprotective agents aimed at suppressing cerebral edema (reviewed in Papadopoulos and Verkman 2007). The importance of AQP4 in edema formation following cerebral ischemia is demonstrated by an up-regulation of AQP4 expression in the endfeet of astrocytes coincident with maximal hemispheric swelling after ischemia (Ribiero and others 2006) and a reduction in edema volume in mice lacking functional AQP4 expression (Manley and others 2000). However, the role of AQP4 in edema formation is not straightforward because mice deficient in AQP4 exhibit substantially worse vasogenic swelling compared with wild-type controls in several models of cerebral injury that induce vasogenic edema (Papadopoulos and others 2004). These results appear to implicate the involvement of AQP4 in the removal of excess fluid from the brain after the onset of vasogenic edema. The role of progesterone in altering brain water content via modulation of AQP4 has not

been fully determined. Studies using TBI models have demonstrated that progesterone administration does reduce brain water content and AQP4 expression (Guo and others 2006). In addition, during pregnancy and the postpartum period in rats (when progesterone levels are elevated), AQP4 expression is increased around intracerebral blood vessels (Quick and Cipolla 2005). Interestingly, a recent study shows that edema formation and AQP4 expression are not affected by gender (Liu and others 2008), and although previous studies have shown that exogenously applied progesterone does reduce edema volume following cerebral ischemia, the effects on AQP4 expression, and function, have yet to be determined.

Neuronal Excitability

At high micromolar concentrations, progesterone inhibits both nicotinic acetylcholine receptor activity (Valera and others 1992; Lena and Changeux 1993) and sigma-1 receptors (reviewed in Maurice and others 2006). Allopregnanolone, a metabolite of progesterone, has been shown to activate $GABA_A$ receptors, enhancing GABAergic neurotransmission (Belelli and Lambert 2005; Mani 2006). The interaction of progesterone and allopregnanolone with $GABA_A$ receptors augments GABAergic neuronal activity, promotes inhibitory neurotransmission, and suppresses the release and function of excitatory neurotransmitters such as glutamate (Brann and others 2005), excesses of which lead to excitotoxicity and subsequently to cell death. In experimental models of TBI, allopregnanolone exhibited anti-apoptotic effects and improved functional recovery (He and others 2004), which is of particular interest given that allopregnanolone was demonstrated to be more effective than progesterone in terms of reducing cortical damage following TBI (Sayeed and others 2006). Progesterone, and its metabolite allopregnanolone, could therefore reduce cell loss by attenuating the development of excitotoxic conditions, although further work is required to determine the involvement of this mechanism after experimental stroke.

Lipid Peroxidation and Oxidative Stress

The neuroprotective effects of progesterone seem to extend also to reducing lipid peroxidation and oxidative stress. Progesterone treatment in rats improved metabolic rates in isolated brain mitochondria, while simultaneously causing a decrease in the release of reactive oxygen species and a reduction in lipid peroxidation, compared with isolated mitochondria from control animals (Irwin and others 2008). In vivo, a decrease in oxidative stress following progesterone treatment has been demonstrated in mice subjected to cerebral ischemia. This suggests that a reduction in oxidative stress is a mechanism of progesterone-mediated neuroprotection (Aggarwal and others 2008) although it may also be a consequence.

Apoptosis and DNA Repair

Progesterone may also modulate apoptotic and DNA repair processes, and consequently improve neuronal survival and neuroregeneration after cerebral injury. Breast cancer studies show that progesterone is capable of both promoting and inhibiting apoptosis. In the CNS, studies utilizing TBI models have indicated that progesterone is able to suppress expression of pro-apoptotic factors such as caspase-3 (Djebaili and others 2004) and nuclear factor-κβ (NF-κβ; Pettus and others 2005) while enhancing the expression of anti-apoptotic factors, such as Bcl-2 (Nilsen and Brinton 2002). Suppression of anti-oxidant release has been demonstrated following progesterone administration using a model of global ischemia suggesting a progesterone-mediated decrease in oxidative stress (Aggarwal and others 2008). Although the effects of progesterone on apoptosis following cerebral ischemia are yet to be determined, factors such as caspase-3 and NF-κβ are important mediators of the cell death that occurs following cerebral ischemia (Schneider and others 1999).

Other Mechanisms of Action

Progesterone and its precursor pregnenolone have been shown to promote myelin production by oligodendrocytes in the CNS (Ghoumari and others 2005) and by Schwann cells in the peripheral nervous system (Koenig and others 1995). Although progesterone has been shown capable of promoting myelin repair following spinal cord injury (Gonzalez and others 2005), its effects upon myelin repair following cerebral ischemia require further clarification (for review, see Schumacher and others 2007).

Conclusion

The long-term goal of experimental studies examining candidate neuroprotective factors is to aid in the development of and inform the design of clinical trials. The concept of neuroprotection is not new, however; what remains unknown is why many agents appear neuroprotective in preclinical models of small-animal ischemia yet none have proven conclusively to be effective in humans. As this review has shown, a large number of experimental studies have established a neuroprotective effect of progesterone for ischemic stroke. In addition, the mechanisms of progesterone action are beginning to be unraveled, although the precise mechanisms of

progesterone's actions following stroke are currently unclear and in this review we have presented those that we consider to be the main possibilities. To date, most of the pharmacological trials of drugs designed to reduce stroke damage have failed. One reason may be that many of these drugs targeted a single aspect of the injury cascade. In contrast, progesterone exerts a multitude of beneficial actions and holds promise as a safe and effective agent for stroke. Thus, it is important to consider what the future direction of study into the relationship between steroid hormones and neuroprotection should take at both a basic and a clinical level.

In terms of experimental studies, it is vital to explore the neuroprotective potential of progesterone in different groups according to hormonal status and age because the majority of experimental studies tend to have focused on using young adult males. Furthermore, as highlighted in this review and a previous systematic review (Gibson and others 2008), researchers should ensure the quality in experimental studies is high, for example, by ensuring randomization and blinding take place to remove any systematic errors of sampling and experimenter bias, respectively. Also, experimental studies need to explore the therapeutic time window of progesterone neuroprotection and evaluate the neuroprotective effect of progesterone when it is administered at time points later than 6 h following the onset of ischemia. It is promising that experimental TBI studies have shown progesterone still to be neuroprotective when administered 24 h following the onset of injury (Roof and others 1996). However, it is still to be determined whether progesterone is effective following experimental stroke after delayed onset of treatment and whether this would translate into positive clinical trials. Drugs such as NXY-059, which did demonstrate a neuroprotective effect following delayed administration (up to 4 h) in experimental stroke models (Sydserff and others 2002), have failed to result in positive clinical trials (Savitz and Schabitz 2008). At the clinical level, progesterone is a potential neuroprotective treatment following cerebral stroke, as demonstrated by experimental evidence. With the success, in terms of safety and improved outcome, of the first two clinical trials of progesterone following TBI (Wright and others 2007; Xiao and others 2008), trials of progesterone administration following cerebral stroke may be a realistic target if subsequent experimental work addresses the issues highlighted here.

References

Aggarwal R, Medhi B, Pathak A, Dhawan V, Chakrabarti A. 2008. Neuroprotective effect of progesterone on acute phase changes induced by partial global cerebral ischaemia in mice. J Pharm Pharmacol 60:731–7.

Alkayed NJ, Harukani I, Kimes AS, London ED, Traystman RJ, Hurn PD. 1998. Gender-linked brain injury in experimental stroke. Stroke 29:159–66.

Alkayed NJ, Traystman RJ, Hurn PD, Miller VM. 2000. Neuroprotective effects of female gonadal steroids in reproductively senescent female rats. Stroke 31:161–8.

Belelli D, Lambert JJ. 2005. Neurosteroids: endogenous regulators of the GABA(A) receptor. Nat Rev Neurosci 6:565–75.

Bingham D, Macrae IM, Carswell HV. 2005. Detrimental effects of 17beta-oestradiol after permanent middle cerebral artery occlusion. J Cereb Blood Flow Metab 25:414–20.

Brann DW, Zamorano PL, De Sevilla L, Mahesh VB. 2005. 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 81:120–8.

Brinton RD, Thompson RF, Foy MR, Baudry M, Wang J, Finch CE, and others. 2008. Progesterone receptors: form and function in brain. Front Neuroendocrinol 29:313–39.

Cai W, Zhu Y, Furuya K, Li Z, Sokabe M, Chen L. 2008. Two different molecular mechanisms underlying progesterone neuroprotection against ischemic brain damage. Neuropharmacology 55:127–38.

Carswell HV, Bingham D, Wallace K, Nilsen M, Graham DI, Dominiczak AF, and others. 2004. Differential effects of 17beta-estradiol upon stroke damage in stroke prone and normotensive rats. J Cereb Blood Flow Metab 24:298–304.

Cervantes M, González-Vidal MD, Ruelas R, Escobar A, Moralí G. 2002. Neuroprotective effects of progesterone on damage elicited by acute global cerebral ischemia in neurons of the caudate nucleus. Arch Med Res 33:6–14.

Chen J, Chopp M, Li Y. 1999. Neuroprotective effects of progesterone after transient middle cerebral artery occlusion in rat. J Neurol Sci 171:24–30.

Choi YC, Lee JH, Hong KW, Lee KS. 2004. 17 b-Estradiol prevents focal cerebral ischemic damage via activation of Akt and CREB in association with reduced PTEN phosphorylation in rats. Fundam Clin Pharmacol 18:547–57.

Coughlan T, Gibson CL, Murphy SP. 2005. Modulatory effects of progesterone on NOS-2 expression in vivo and in vitro. J Neurochem 93:932–42.

Cutler SM, VanLandingham JW, Stein DG. 2006a. Tapered progesterone withdrawal promotes long-term recovery following brain trauma. Exp Neurol 200:378–85.

Cutler SM, VanLandingham JW, Murphy AZ, Stein DG. 2006b. Slow-release and injected progesterone treatments enhance acute recovery after traumatic brain injury. Pharmacol Biochem Behav 84:420–8.

Djebaili MS, Hoffman SW, Stein DG. 2004. Allopregnanolone and progesterone decrease cell death and cognitive deficits after a contusion of the rat pre-frontal cortex. Neuroscience 123:349–59.

Donnan GA, Fisher M, Macleod M, Davis SM. 2008. Stroke. Lancet 371:1612–23.

Gebel JM, Jauch EC, Brott TG, Khoury J, Sauerbeck L, Salisbury S, and others. 2002. Relative edema volume is a predictor of outcome in patients with hyperacute spontaneous intracerebral hemorrhage. Stroke 33:2636–41.

Ghoumari AM, Baulieu EE, Schumacher M. 2005. Progesterone increases oligodendroglial cell proliferation in rat cerebellar slice cultures. Neuroscience 135:47–58.

Gibson CL, Constantin DC, Prior MJW, Bath PMW, Murphy SP. 2005. Progesterone suppresses the inflammatory response and nitric oxide synthase-2 expression following cerebral ischemia. Exp Neurol 193:522–30.

Gibson CL, Gray LJ, Bath PMW, Murphy SP. 2008. Progesterone for the treatment of experimental brain injury: a systematic review. Brain 131:318–28.

Gibson CL, Gray LJ, Murphy SP, Bath PMW. 2006. Estrogens and experimental ischemic stroke: a systematic review. J Cereb Blood Flow Metab 26:1103–13.

Gibson CL, Murphy SP. 2004. Progesterone enhances functional recovery following middle cerebral artery occlusion in male mice. J Cereb Blood Flow Metab 24:805–13.

Ginsberg MD. 2009. Current status of neuroprotection for cerebral ischaemia, synoptic overview. Stroke 40:S111–4.

Gonzalez SL, Labombarda F, Deniselle MC, Mougel A, Guennoun R, Schumacher M, and others. 2005. Progesterone neuroprotection in spinal cord trauma involves up-regulation of brain-derived neurotrophic factor in motoneurons. J Steroid Biochem Mol Biol 94:143–9.

González-Vidal MD, Cervera-Gaviria M, Ruelas R, Escobar A, Moralí G, Cervantes M. 1998. Progesterone: protective effects on the cat hippocampal neuronal damage due to acute global cerebral ischemia. Arch Med Res 29:117–24.

Green AR, Odergren T, Ashwood T. 2003. Animal models of stroke: do they have value for discovering neuroprotective agents? Trends Pharmacol Sci 24:402–8.

Guennoun R, Meffre D, Labombarda F, Gonzalez SL, Deniselle MC, Stein DG, and others. 2008. The membrane-associated progesterone-binding protein 25-Dx: expression, cellular localization and up-regulation after brain and spinal cord injuries. Brain Res Rev 57:493–505.

Guo Q, Sayeed I, Baronne LM, Hoffman SW, Guennoun R, Stein DG. 2006. Progesterone administration modulates AQP4 expression and edema after traumatic brain injury in male rats. Exp Neurol 198:469–78.

Hattori K, Lee H, Hurn PD, Crain BJ, Traystman RJ, DeVries AC. 2000. Cognitive deficits after focal cerebral ischemia in mice. Stroke 31:1939–44.

He J, Evans CO, Hoffman SW, Oyesiku NM, Stein DG. 2004. Progesterone and allopregnanolone reduce inflammatory cytokines after traumatic brain injury. Exp Neurol 189:404–12.

Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, and others; the HERS Research Group. 1998. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. JAMA 280:605–11.

Hurn PD, Macrae IM. 2000. Estrogen as a neuroprotectant in stroke. J Cereb Blood Flow Metab 20:631–52.

Hurn PD, Aardelt AA, Alkayed NJ, Crain BJ, Hu W, Kearney ML, and others. 2002. Estrogen and testosterone as neuroprotectants in stroke. In: Krieglstein J, editor. Pharmacology of cerebral ischemia 2002. Stuttgart: Medpharm Scientific Publishers. p 17–24.

Irwin RW, Yao J, Hamilton RT, Cadenas E, Brinton RD, Nilsen J. 2008. Progesterone and estrogen regulate oxidative metabolism in brain mitochondria. Endocrinology 149: 3167–75.

Jiang N, Chopp M, Stein D, Feit H. 1996. Progesterone is neuroprotective after transient middle cerebral artery occlusion in male rats. Brain Res 735:101–7.

Koenig HL, Schumacher M, Ferzaz B, Thi AN, Ressouches A, Guennoun R, and others. 1995. Progesterone synthesis and myelin formation by Schwann cells. Science 268:1500–3.

Kumon Y, Kim SC, Tompkins P, Stevens A, Sakaki S, Loftus CM. 2000. Neuroprotective effect of postischemic administration of progesterone in spontaneously hypertensive rats with focal cerebral ischemia. J Neurosurg 92:848–52.

Lena C, Changeux JP. 1993. Allosteric modulations of the nicotinic acetylcholine receptor. Trends Neurosci 16: 181–6.

Liu X, Zhang W, Alkayed NJ, Froehner SC, Adams ME, Amiry-Moghaddam M, and others. 2008. Lack of sex-linked differences in cerebral edema and aquaporin-4 expression after experimental stroke. J Cereb Blood Flow Metab 28:1898–1906.

Lo EH, Dalkara T, Moskowitz MA. 2003. Mechanisms, challenges and opportunities in stroke. Nat Rev Neurosci 4:399–415.

Macleod MR, O'Collins T, Horky LL, Howells DW, Donnan GA. 2005. Pooling of animal experimental data reveals influence of study design and publication bias. J Cereb Blood Flow Metab 25:713–21.

Macrae IM, Carswell HV. 2006. Oestrogen and stroke: the potential for harm as well as benefit. Biochem Soc Trans 34:1362–5.

Mani SK. 2006. Signaling mechanisms in progesterone-neurotransmitter interactions. Neuroscience 138:773–81.

Manley GT, Fujimura M, Ma T, Noshita N, Filiz F, Bollen AW, and others. 2000. Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. Nat Med 6:159–63.

Maurice T, Gregoire C, Espallergues J. 2006. Neuro(active)steroids actions at the neuromodulatory sigma1 (sigma1) receptor: biochemical and physiological evidences, consequences in neuroprotection. Pharmacol Biochem Behav 84:581–97.

Meffre D, Delespierre B, Gouezou M, Leclerc P, Vinson GP, Schumacher M, Stein and others. 2005. 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 93:1314–26.

Moralí G, Letechipía-Vallejo G, López-Loeza E, Montes P, Hernández-Morales L, Cervantes M. 2005. Post-ischemic administration of progesterone in rats exerts neuroprotective effects in the hippocampus. Neurosci Lett 382:286–90.

Murphy SJ, Traystman RJ, Hurn PD. 2000. Progesterone exacerbates striatal stroke injury in progesterone-deficient animals. Stroke 31:1173–8.

Murphy SJ, Littleton-Kearney MT, Hurn PD. 2002. Progesterone administration during reperfusion, but not preischemia alone, reduces injury in ovariectomised rats. J Cereb Blood Flow Metab 22:367–88.

Murphy SJ, McCullough LD, Smith JM. 2004. Stroke in the female: role of biological sex and estrogen. ILAR J 45:147–59.

Niermann H, Amiry-Moghaddam M, Holthoff K, Witte OW, Ottersen OP. 2001. A novel role of vasopressin in the

brain: modulation of activity-dependent water flux in the neocortex. J Neurosci 21:3045–51.
Nilsen J, Brinton RD. 2002. Impact of progestins on estrogen-induced neuroprotection: synergy by progesterone and 19-norprogesterone and antagonism by medroxyprogesterone acetate. Endocrinology 143:205–12.
Papadopoulos MC, Manley GT, Krishna S, Verkman AS. 2004. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. FASEB J 18:1291–3.
Papadopoulos MC, Verkman AS. 2007. Aquaporin-4 and brain edema. Pediatr Nephrol 22:778–84.
Pettus EH, Wright DW, Stein DG, Hoffman SW. 2005. Progesterone treatment inhibits the inflammatory agents that accompany traumatic brain injury. Brain Res 1049: 112–9.
Prencipe M, Ferretti C, Casini AR, Santini M, Giubilei F, Culasso F. 1997. Stroke, disability and dementia: results of a population survey. Stroke 28:531–6.
Quick AM, Cipolla MJ. 2005. Pregnancy-induced upregulation of aquaporin-4 protein in brain and its role in eclampsia. FASEB J 19:170–5.
Rash JE, Yasumura T, Hudson CS, Agre P, Nielsen S. 1998. Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. Proc Natl Acad Sci USA 95:11981–6.
Reglodi D, Tamas A, Lengvari I. 2003. Examination of sensorimotor performance following middle cerebral artery occlusion in rats. Brain Res Bull 59:459–66.
Ribiero C, Hirt L, Bogousslavsky J, Regli L, Badaut J. 2006. Time course of aquaporin expression after transient focal cerebral ischemia in mice. J Neurosci Res 83: 1231–40.
Rogers DC, Campbell CA, Stretton JL, Mackay KB. 1997. Correlation between motor impairment and infarct volume after permanent and transient middle cerebral artery occlusion in the rat. Stroke 28:2060–6.
Roof RL, Duvdevani R, Heyburn JW, Stein DG. 1996. Progesterone rapidly decreases brain edema: treatment delayed up to 24 hours is still effective. Exp Neurol 138:246–51.
Sacco RL, Benjamin EJ, Broderick JP, Dyken M, Easton JD, Feinberg WM, and others. 1997. American heart association prevention conference IV: prevention and rehabilitation of stroke: risk factors. Stroke 28:1507–17.
Savitz SI, Schabitz WR. 2008. A critique of SAINT II: wishful thinking, dashed hopes, and the future of neuroprotection for acute stroke. Stroke 39:1389–91.
Sayeed I, Guo Q, Hoffman SW, Stein DG. 2006. Allopregnanolone, a progesterone metabolite, is more effective than progesterone in reducing cortical infarct volume after transient middle cerebral artery occlusion. Ann Emerg Med 47:381–9.
Sayeed I, Wali B, Stein DG. 2007. Progesterone inhibits ischemic brain injury in a rat model of permanent middle cerebral artery occlusion. Restor Neurol Neurosci 25:151–9.
Schneider A, Martin-Villalba A, Weih F, Vogel J, Wirth T, Schwaninger M. 1999. NF-kappaB is activated and promotes cell death in focal cerebral ischemia. Nat Med 5:554–9.
Schumacher M, Guennoun R, Stein DG, De Nicola AF. 2007. Progesterone: therapeutic opportunities for neuroprotection and myelin repair. Pharmacol Ther 116:77–106.
Simon JA, Hsia J, Cauley JA, Richards C, Harris F, Fong J, and others; the HERS Research Group. 2001. Postmenopausal hormone therapy and risk of stroke: the Heart and Estrogen-Progestin Replacement Study (HERS). Circulation 103: 638–42.
Singh M. 2006. Progesterone-induced neuroprotection. Endocrine 29:271–4.
Stein DG, Wright DW, Kellermann AL. 2008. Does progesterone have neuroprotective properties? Ann Emerg Med 51:164–72.
Stein DG. 2005. The case for progesterone. Ann N Y Acad Sci 1052:152–69.
Stroke Therapy Academic Industry Roundtable (STAIR). 1999. Recommendations for standards regarding preclinical neuroprotective and restorative drug development. Stroke 30:2752–8.
Sydserff SG, Borellie AR, Green AR, Cross AJ. 2002. Effects of NXY-059 on infarct volume after transient or permanent middle cerebral artery occlusion in the rat; studies on dose, plasma concentration and therapeutic time window. Br J Pharmacol 135:103–12.
Toung TJ, Chen TY, Littleton-Kearney MT, Hurn PD, Murphy SJ. 2004. Effects of combined estrogen and progesterone on brain infarction in reproductively senescent female rats. J Cereb Blood Flow Metab 24:1160–6.
Toung TJ, Hurn PD, Traystman RJ, Sieber FE. 2000. Estrogen decreases infarct size after temporary focal ischaemia in a genetic model of type I diabetes mellitus. Stroke 31:2701–6.
Turgeon JL, McDonnell, Martin KA, Wise PM. 2004. Hormone therapy: physiological complexity belies therapeutic simplicity. Science 304:1269–73.
Turtzo LC, McCullough LD. 2008. Sex differences in stroke. Cerebrovasc Dis 26:462–74.
Valera S, Ballivet M, Bertrand D. 1992. Progesterone modulates a neuronal nicotinic acetylcholine receptor. Proc Natl Acad Sci USA 89:9949–53.
Viscoli CM, Brass LM, Kernan WN, Sarrel PM, Makuch R, Horwitz RJ. 2001. A clinical trial of estrogen-replacement therapy after ischemic stroke. N Engl J Med 345:1243–9.
Wassertheil-Smoller S, Hendrix SL, Limacher M, Heiss G, Kooperberg C, Baird A, and others; WHI Investigators. 2003. Effect of estrogen plus progestin on stroke in postmenopausal women—the Women's Health Initiative: a randomized trial. JAMA 289:2673–4.
Wenger NK, Speroff L, Packard B. 1993. Cardiovascular health and disease in women. N Engl J Med 329:247–56.
Wise PM, Dubal DB, Wilson ME, Rau SW, Bottner M, Rosewell KL. 2001. Estradiol is a neuroprotective factor in the adult and aging brain: understanding of mechanisms derived from in vivo and in vitro studies. Brain Res Brain Res Rev 37:313–9.
Wright DW, Kellerman AL, Hertzberg VS, Clark PL, Frankel M, Goldstein FC, and others. 2007. ProTECT: a randomized clinical trial of progesterone for acute traumatic brain injury. Ann Emerg Med 49:391–402.
Xiao G, Wei J, Yan W, Wand W, Lu Z. 2008. Improved outcomes from the administration of progesterone to patients with acute severe traumatic brain injury: a randomized controlled trial. Crit Care 12:R61.