CMLS, Cell. Mol. Life Sci. 62 (2005) 271–280
1420-682X/05/030271-10
DOI 10.1007/s00018-004-4382-2

CMLS Cellular and Molecular Life Sciences

Estrogens, progestins, menopause and neurodegeneration: basic and clinical studies

J. W. Simpkins *, S.-H. Yang, Y. Wen and M. Singh

Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, Texas 76107 (USA), e-mail: jsimpkin@hsc.unt.edu

Abstract. Two classes of ovarian steroids, estrogens and progestins, are potent in protecting neurons against acute toxic events as well as chronic neurodegeneration. Herein we review the evidence for neuroprotection by both classes of steroids, provide plausible mechanisms for these potent neuroprotective activities and indicate the need for further clinical trials of both estrogens and progestins in protection against acute and chronic conditions that cause neuronal death. Estrogens at concentrations ranging from physiological to pharmacological are neuroprotective in a variety of in vitro and in vivo models of cerebral ischemia and brain trauma as well as in reducing key neuropathologies of Alzheimer's disease. While the mechanisms of this potent neuroprotection are currently unresolved, a mitochondrial mechanism is involved. Progestins have been recently shown to activate many of the signaling pathways used by estrogens to neuroprotect, and progestins have been shown to protect against neuronal loss in vitro and in vivo in a variety of models of acute insult. Collectively, results of these animal and tissue culture models suggest that the loss of both estrogens and progestins at the menopause makes the brain more vulnerable to acute insults and chronic neurodegenerative diseases. Further clinical assessment of appropriate regimens of estrogens, progestins and their combination are supported by these data.

Key words. Estrogens; progestins; neuroprotection; stroke; Alzheimer's disease; ischemia; estrogen receptor.

Introduction

Strokes are the leading cause of neurological disability and a major cause of death, with over 750,000 new strokes per year in the U.S. alone [1, 2]. Despite this, there is currently only one available therapy that attempts to reduce the damaging effects of stroke, the anticoagulant, tissue plasminogen activator (t-PA) [3–5]. Desperately needed, but currently unavailable are therapies that can prevent or reduce brain damage during cerebral ischemia. In addition, Alzheimer's disease (AD) is the leading cause of dementia, currently affecting 4 million Americans, and it is estimated that more than 14 million will be affected by 2030 [6]. No current therapies can effectively slow the progression or prevent this devastating neurodegenerative disease.

In the present review, we evaluate the literature and discuss our own data related to the potential use of estrogens and progestins in neuroprotection during cerebral ischemia as well as in AD. We believe that there is now a compelling scientific rationale for initiation of clinical trials of estrogens and progestins for the treatment of brain damage related to cerebral ischemia, and there are currently ongoing and proposed trials for estrogens and progestins in AD prevention. Here, we review the basic and clinical literature indicating the potential of acute estrogen and progestin administration as a neuroprotective therapy for ischemia and chronic estrogen and/or progestin therapy as a potential preventative of AD.

Current Clinical Uses of Estrogens

We have better knowledge of the pharmacology and toxicity of estrogen than any other class of drugs. Estrogens are used in therapies aimed at prevention of pregnancy as a component of birth control pills [7], in postmenopausal estrogen therapy (ET) or as an essential component of postmenopausal hormone therapy (HT) [7], and for use in the treatment of a few kinds of otherwise untreatable can-

* Corresponding author.

cers [7]. Estrogens and progestins as the components of birth control pills, ET and HT are among the most prescribed compounds known with literally millions of prescriptions written each day. Additionally, there are hundreds of millions of women years' worth of experience in the use of estrogens and progestins in postmenopausal ET and HT.

Dozens of preparations of birth control pills are currently marketed in the U.S. All of these preparations use synthetic estrogens and progestins, as naturally occurring estrogens and progestins are extensively metabolized in the first pass through the liver and therefore are not readily bioavailable. In the U.S. the most commonly used ET is Premarin®, a conjugated equine estrogen preparation, and the most common HT is PremPro®, a combination of Premarin and medroxyprogesterone acetate.

Evidence for efficacy of estrogens and estrogen analogues in stroke neuroprotection

A possible role for endogenous female hormones as neuroprotectants against global cerebral ischemia-reperfusion injury was suggested by studies demonstrating that intact adult female rodents sustain less neuronal damage as compared to age-matched males [8]. Our laboratory first demonstrated that 17β-estradiol (17β-E2) is a potent neuroprotectant in vitro [8] and is very effective against ischemia-induced brain damage [9]. There is now abundant evidence for neuroprotection by estrogens both in vitro and in vivo.

Protective effects of estrogen have been widely reported in a variety of neurons against different toxicities, which mimic cerebral ischemia in vitro, including serum deprivation, oxidative stress and excitotoxicity [10]. Multiple independent and lethal mechanisms are involved in cerebral ischemia-induced neuronal death. Estrogens have been identified as multi-faceted hormones that antagonize many aspects of damage-inducing cascades resulting from cerebral ischemia. Antioxidant effects of the steroid [11] and attenuation of N-methyl-D-aspartate (NMDA) receptor activation [12] have been implicated as mechanisms for the neuroprotective effects of estrogens. Also, two of the major signaling pathways, ERK and PI-3K-Akt, have been well characterized as being able to mediate inhibition of apoptosis and support neuronal survival. Both signal pathways have been demonstrated to be activated by estrogens [13, 14] and more recently by progestins (see below).

The mitochondria have become a focus of studies to discover mechanisms and new drugs for the treatment of neurodegeneration. Mitochondria produce most of the cellular ATP by oxidative phosphorylation and generate most of the endogenous oxygen radicals as a toxic by-product. In addition, mitochondria are central in the regulation of apoptosis, calcium homeostasis and cellular redox state. Several studies have also shown that estrogens may exert direct or indirect effects on mitochondrial function. Estradiol can protect against ATP depletion, mitochondrial membrane potential decline and the generation of reactive oxygen species, induced by the mitochondrial toxin 3-nitroproprionic acid [15] and the pro-oxidant, H_2O_2 [X. Wang and J. W. Simpkins, unpublished observations]. 17β-Estradiol can modulate mitochondrial calcium influx and increase expression of Bcl-2, an anti-apoptotic protein whose major site of action is the mitochondria [16]. As a possible substrate for estrogen's actions on the mitochondria, we have recently demonstrated that estrogen receptor β (ERβ) localizes to the mitochondria in a variety of cell types [17, 18].

In in vivo studies, the neuroprotective effects of estrogens have been demonstrated in a variety of models of acute cerebral ischemia. These include transient and permanent middle cerebral artery occlusion models [9, 19, 20], global forebrain ischemia models [21], photothrombotic focal ischemia models [22] and glutamate-induced focal cerebral ischemia models [23]. The protective effects of estrogens have been described in rats, mice and gerbils [9, 24, 25]. Estrogen-induced neuroprotection has been demonstrated in adult female, middle-aged female as well as reproductively senescent female rats [26]. Similarly, these effects of estrogens have been shown despite the presence of diabetes and hypertension [27, 28]. The neuroprotective effects of estrogens also have been demonstrated against subarachnoid hemorrhage, a highly prevalent form of stroke in females [29]. Finally, these protective actions of estrogen are not limited to the female, but have also been seen in males [30, 31]. Collectively, these results indicate that estrogens could be valuable candidates for brain protection during acute stroke in both males and females.

The concentrations of estrogens, ranging from low physiological to high pharmacological, have been shown to produce protective effects in stroke models. No neuroprotection was afforded by the administration of physiological level of estradiol at the onset of an ischemic event [20], but neuroprotective effects of pharmacological doses of estradiol were clearly demonstrated by the acute treatment at the time of or just before an ischemic event, as well as after its onset [9, 31, 32]. The therapeutic window of estrogens at the dose of 100 µg/kg lasts up to 3 h after insult [33], and this therapeutic window can be extended to up to 6 h after ischemic insult with doses of 500–1000 µg/kg [34]. This long post-event efficacy of estrogens is promising, since the therapeutic window for estrogen neuroprotection could be insult severity dependent and could vary between different species. It has been shown that the infarct penumbra, which can be protected, develops over a longer period in human subjects than in rodents [35]. So it is reasonable to predict that estrogens

could have a longer therapeutic window in human than the 6-h window that we have described in rodents.

Inasmuch as ERs are distributed throughout central nervous system (CNS), three possible mechanisms could contribute to the neuroprotective action of estrogens in vivo: (i) a genomic estrogen receptor-mediated action, (ii) a receptor-dependent nongenomic action such as pro-survival signaling activation and/or (iii) a receptor-independent nongenomic action. Genomic actions of estrogens are mediated by binding of the steroid to the nuclear ERs, dimerization of these receptor-ligand complexes and the binding of these dimers to the ER response element, thereby activating transcriptional events. There is growing evidence that indicates nongenomic steroid action as well. Nongenomic actions are principally characterized by their rapid onset of action and insensitivity to inhibitors of transcription and protein synthesis [36]. The different therapeutic windows for physiological and pharmacological doses of estrogens suggest that different doses afford neuroprotective action through different mechanisms.

It has become evident that estrogens as well as nonfeminizing estrogen analogues exert neuroprotective influences, indicating that these effects of estrogens do not require ER-dependent gene transcription. 17β-E2 as well as nonfeminizing estrogen analogues, such as 17α-E2 and the complete enantiomer of 17β-E2, ENT-E2, can preserve mitochondrial function, cell viability and ATP levels in human lens cells during oxidative stress, an effect that is not blocked by ICI182,780, an estrogen receptor antagonist [37]. Surprisingly, ICI182,780 alone increases cell survival [37]. Several other synthesized estrogen analogues also have been reported to possess neuroprotective properties [38–40]. Structure-activity relationship studies indicate that the most essential structural motif for estrogen's neuroprotective functions is the phenolic A ring of the steroid, rather than its ability to bind to ERs [38].

ERα plays an important role in reproductive development and function in both females and males [41]. Although ERs have been found throughout the CNS, no grossly observable phenotypic change in the brain have been found in either ERα or ERβ knockout mice, suggesting that effects of estrogens on these two known ERs have relatively little role in CNS development [42]. The rapid onset of the neuroprotective action induced by estrogens indicates that these effects are unlikely mediated through genomic mechanisms. The neuroprotective actions against stroke have been demonstrated not to be limited to estrogens, but are also seen with selective estrogen receptor modulators, such as tamoxifen [43, 44] and LY353381 [45]. Further, neuroprotective actions of other non-feminizing estrogen analogues, such as 17α-E2 [9], the complete enantiomer of 17β-E2 [40], and an adamantyl estrogen analogue [46], also have been demonstrated in stroke models. The actions of these non-receptor-binding estrogen analogues indicated that ERs are not required for the neuroprotective effects of estrogens.

Given the expected clinical safety of acutely administered estrogens, and the plethora of data supporting a neuroprotective role of estrogens against ischemic stroke, estrogen therapy may be useful in treating acute cerebral ischemia. Further, the efficacy of non-feminizing estrogen analogues suggests that these compounds may be clinically useful for treating neuronal death in men or women for whom estrogen therapy is contraindicated. Also, the long therapeutic window of estrogens makes them candidates for affording neuroprotection in ongoing strokes.

Other neuroprotective agents have been developed which target specific components of the ischemic cascade. However, the results to date of the neuroprotective clinical trials have been discouraging, suggesting that the strategy of applying a single drug that interferes with a specific event in the ischemic cascade will not have a large clinical impact [47]. Protection of neurons during ischemia/reperfusion will require a polypharmacy approach or use of a compound that has pleotrophic actions and appears to affect multiple neurotoxic processes. It is encouraging that estrogens have proven to be multifaceted hormones modulating many aspects of neuronal cascades induced by cerebral ischemia. The pathological mechanisms that are activated during stoke, including oxidative stress, free radical activity, excitotoxicity, inflammatory response, mitochondrial dysfunction and apoptosis, are antagonized by estrogens. The protective effects of estrogens during reperfusion made them the candidates of neuroprotectants against reperfusion injury induced by thrombolysis, an outcome that could prolong the therapeutic time window for successful thrombolysis by administration of estrogens before, during, or after the infusion of t-PA. This strategy should be effective over the short term in producing pharmacotherapies that can reduce ischemic brain damage and the resulting neurological deficits.

Progestin biology

Progesterone, the natural progestin, is a major gonadal hormone that is synthesized primarily by the ovary (corpus luteum) in the female, and the testes and adrenal cortex in the male. While progesterone levels are generally higher in the female, it is worth noting that levels of progesterone during the female follicular phase of the menstrual cycle are similar to those seen in males [48], suggesting that progesterone may play an equally important role in both sexes. The paradigmatic mechanism by which progesterone elicits its effects is via the progesterone receptor (PR), which like the ER, has classically been described as a nuclear transcription factor, acting through specific progesterone response elements (PREs) within

the promoter region of target genes to regulate transcription. Two major isoforms of the classical progesterone receptor exist, PR-B, and its N-terminally truncated form, PR-A (for review, see [49]). The latter has been shown to exert negative control of not only PR-B-mediated transcription, but that mediated by the ER and glucocorticoid receptor as well [50]. This negative regulation of ER function by a PR may underlie, at least in part, the mechanism by which progestins functionally antagonize the effects of estrogen. For example, progestins inhibit estrogen's ability to increase serum levels of 1,25-dihydroxy vitamin D [51], whose consequence may be to antagonize estrogen's beneficial effects on the bone. However, the interaction between the two receptors may not only result in transrepression, but may also be cooperative in nature. For example, Migliaccio et al. demonstrated a physical interaction of the progesterone receptor with the estrogen receptor, and this association was necessary for progesterone to elicit the activation of the mitogen-activated protein kinase (MAPK) pathway in mammary tumor cells [52].

As introduced by the last statement, progesterone can also elicit its effects via non-genomic mechanisms (such as the activation of typically growth factor-associated signal transduction pathways). The growing list of second messenger/signal transduction systems activated by progesterone include cAMP/PKA [53], MAPK (ERK1/2) [52, 54] and the PI-3K/Akt pathway [54]. Activation of such signaling pathways may not only be relevant to how progesterone regulates cellular function related to reproductive function, but may also be an important mechanism by which progesterone elicits its neuroprotective effects. As mediators of these non-genomic effects, novel receptor systems for progesterone have been suggested. For example, progesterone may exert its effects via interactions with membrane binding sites, characterized in the brain by the demonstration of specific, displaceable binding in synaptosomal membrane preparations [55, 56]. Such membrane binding sites may include the recently cloned membrane progesterone receptor that exhibits characteristics of G-protein coupled receptors [57, 58]. Progesterone, through its metabolites, can also interact with membrane-associated receptors coupled to ion channels, such as the gamma aminobutyric acid A ($GABA_A$) receptor system (see [59] for review). Such metabolites include allopreganolone (or $3\alpha,5\alpha$-tetrahydroprogesterone), which can bind to discrete sites within the hydrophobic domain of the $GABA_A$ receptor complex, and result in the potentiation of GABA-induced chloride conductance, and in turn may regulate cellular excitability and thus, excitotoxicity. Thus, progesterone's ability to interact with specific sites within the membrane [either membrane binding sites (receptors) or with the $GABA_A$ receptor], as well as with specific cytosolic signal transducers, may help explain some of the rapid effects of progesterone, which in addition to its classical genomic mechanisms may be important for regulating cell viability.

Progestin pharmacology and current clinical uses

The major form of progestin used in HT is the synthetic compound medroxyprogesterone acetate (MPA), which is the major progestin used in the formulation of hormone therapy and oral contraceptives. With regards to HT, these regimens include an added progestin in order to counteract a perceived increase risk of certain cancers such as endometrial cancer resulting from unopposed estrogen treatment (for review, see [60]). The natural hormone progesterone (Prometrium) is also used, though to a lesser degree in the U.S. While both the synthetic progestins and the natural hormone, progesterone, can elicit similar effects [i.e. both can inhibit the uterotrophic effects of estrogen and can exert an inhibitory influence (negative feedback) on gonadotropin secretion at the level of the hypothalamus], it is important to recognize that these hormones do not always elicit the same response. For example, progesterone has been described to be neuroprotective [61, 62], whereas the synthetic progestin MPA was not [62]. Moreover, MPA antagonized the effects of estrogen, while the natural hormone progesterone did not [63, 64]. Such differences may be important in considering the results of the recently published Women's Health Initiative (WHI) studies which used MPA rather than progesterone and, further, could provide critical insight into the development of the most effective therapeutic formulations for the treatment of various post-menopausal conditions.

Progestins and neuroprotection

A considerable amount of information has been obtained regarding the mechanisms underlying estrogen's protective effects. One experimental model that has been valuable in the validation of the hypothesis that estrogens are beneficial is the use of the ovariectomized animal. Ovariectomy results in impaired cellular function that is reflected by behavioral, neurochemical and molecular deficits consistent with those seen with advanced age or in certain age-associated diseases such as AD. Estrogen treatment of ovariectomized animals at least partially normalizes the deficits [65–68]. It is important to recognize, however, that ovariectomy results not only in the loss of the primary forms of circulating estrogen, but also in the loss of another major ovarian hormone, progesterone. Thus, the behavioral, neurochemical and molecular deficits that result from ovariectomy may not only be due to a loss in circulating estrogen levels, but may also be a consequence of progesterone loss. Moreover, estrogen-

replacement does not always lead to complete recovery of the ovariectomy-induced deficit [68]. As such, this partial normalization could be a result of not having replaced the other steroid hormones similarly lost following ovariectomy.

In humans, the menopause is also characterized by the concomitant loss of progesterone, and not just estrogen. As such, the increased risk for developing Alzheimer's disease may be contributed by the precipitous decline in both estrogen and progesterone levels. Thus, it is possible that progesterone may be equally beneficial, either alone or in conjunction with estrogen. In fact, progesterone, like estrogen, has been reported to have neuroprotective effects in various experimental models. In hippocampal neurons, both estradiol and progesterone were shown to reduce neuronal vulnerability to such insults as glutamate, $FeSO_4$ and Aβ toxicity [61]. In addition, secondary neuronal loss following cortical contusion injury and resulting cognitive impairment was significantly reduced in mice that received progesterone treatment relative to untreated controls [69, 70]. Progesterone was also effective at reducing the amount of cell death seen in an acute model of global ischemia [71]. Moreover, progesterone has been shown to be protective against excitotoxic insult and promote morphological and functional recovery in the Wobbler mouse, an animal model of spinal cord degeneration [72].

Mechanistically, progesterone-induced neuroprotection may be mediated by the $GABA_A$ receptor. Progesterone's metabolites can, in fact, bind to a site within the $GABA_A$ receptor complex and, as a consequence, potentiate the effect of GABA on its receptor ([59] for review). The activation of the $GABA_A$ receptor in turn has been shown to modulate cell survival, particularly in models of excitotoxicity and may be consistent with the protective effect of progesterone seen against kainite-induced seizure activity and subsequent cell death [73]. Also, progesterone has been described to have antioxidant effects [74] that may also contribute to neuronal survival following injury. Alternatively, progesterone may be protective through its ability to elicit the activation of specific signaling pathways relevant to neuroprotection [54, 62] as well as increasing the expression of anti-apoptotic proteins such as Bcl-2 [62]. Collectively, there is growing evidence that supports the importance of progesterone, either alone or in combination with estrogen, in promoting cell survival.

Transient cerebral ischemia as a model for AD neuropathology

There is an increased prevalence of dementia among stroke patients. In individuals who are over 60 years of age and suffer a stroke, the prevalence of dementia is ninefold higher than controls at 3 months after an ischemic attack [75]. Among patients who had their first cerebral infarct without previous dementia, the incidence of dementia in the first year is nine times greater than expected [76]. Four years after a first lacunar infarct, 23% of patients develop dementia, 4–12 times more than controls [77]. Brain damage from the initial infarct is considered as the direct cause of less than 50% of the dementia [75], and many of the post-stroke dementias have a progressive onset, suggesting a degenerative process rather than a vascular event [76, 78, 79].

AD is the most frequent cause of degenerative dementia [80]. Interestingly, AD and stroke have many common risk factors. The e4 allele of the apolipoprotein E gene (*APOE 4*) is associated with a higher risk of ischemic stroke, coronary heart diseases [81, 82] and dementia [82, 83]. The *APOE* e*4* allele also has been firmly established as a major risk factor for late-onset AD [81, 84, 85]. It is also known that amyloid precursor protein (AβPP) accumulates in regions of neurodegeneration following focal cerebral ischemia in the rat [86, 87]. AD-type pathology is often associated with cerebral amyloid angiopathy [88] and with infarcts [89]. Alz-50-immunoreactive granules are found around cerebral infarction after a stroke [90]. Therefore, the link between stroke and AD seems to be closer than can be explained by chance.

Apoptosis also links neurodegenerative diseases and stroke. Studies of the pathogenic mechanisms implicate apoptosis in age-related neurological disorders including AD and Parkinson's disease [91–93]. The pathology involves synaptic degeneration, senile plaques, neurofibrillary tangles (NFTs) and death of neurons in limbic structures, including the hippocampus and the cerebral cortex. Substantial apoptosis was identified in AD and other neurodegenerative diseases in neurons containing NFTs, and associated with amyloid deposits [94, 95]. Similarly, focal ischemic stroke results in brain damage with an ischemic core region and a surrounding ischemic penumbra [96]. A number of pro-apoptotic factors appear to serve a similar role in promoting neuronal death, which includes overactivation of glutamate receptors, calcium overload and increased reactive oxygen species. In addition, complex cytokine cascades involving microglial activation and the cerebrovasculature are implied in both diseases.

We found that transient focal ischemia induces a profound hyperphosphorylation and a conformational change in tau, both of which are similar to the tau hyperphosphorylation and NFTs seen in AD brains [97]. Additionally, 17β-estradiol substantially reduces tau phospho-epitopes as defined by a variety of antibodies in the ischemic cortex [97].

The relationship between ischemic stroke and the formation of NFTs is now the subject of intense investigation in our laboratory. Emerging evidence from signal transduction and biochemical studies suggests that a neuronal cdc2-like kinase, cyclin-dependent kinase 5 (cdk5), may

be involved in the effects of ischemic stroke on NFT formation. Our data support the model for NFT formation following ischemia/reperfusion shown in figure 1. Ischemia/reperfusion causes a variety of pro-apoptotic events, including glutamate-induced excitotoxicity and severe oxidative stress. These neurotoxic events lead to an influx of extracellular Ca^{2+} and a release of intracellular Ca^{2+} stores. This results in elevated calpain activity. Activated calpain cleaves p35 to p25, which confers potent neurotoxicity in neurons and may lead to sustained deregulation of cdk5 in neurons [47, 98, 99]. The resulting alteration in kinase activities ultimately leads to the hyperphosphorylation of tau and eventually formation of NFTs [100].

This hypothesis is consistent with the observations that Aβ peptide production and pro-oxidative events, such as ischemia/reperfusion, disrupt neuronal metabolic and ionic homeostasis and cause aberrant activation of kinases and/or inhibition of phosphatases. The resulting alteration in kinase and phosphatase activities ultimately leads to hyperphosphorylation of tau and formation of NFTs. Cdk5 is a tau kinase that can be induced by Aβ peptides or ischemic damage. Its deregulation may represent one of the signal transduction pathways that connect Aβ toxicity to tau hyperphosphorylation.

The WHI studies

The recent publication of the results from the WHI studies, which tested PremPro® as the combined HT have been widely interpreted as evidence against the use of combined estrogen/progestin therapies for the treatment of AD. Rather than the expected decrease in incidence of AD, an increase in 'all cause dementias' was reported in the PremPro® group [101, 102]. Regrettably, the trial has been grossly overinterpreted, as it was a test of a single formulation of equine estrogens and medroxyprogesterone acetate in an elderly population of women who likely had underlying cardiovascular disease [103]. The trial enrolled women who were at an average age of 63 years and treated these older women with PremPro® continuously. As such, these women had experienced, on average, a decade without exposure to ovarian hormones, and then were suddenly exposed to continuous steroids orally, a very unphysiological exposure regimen. It is well known that once plaques are formed, they are destabilized by estrogen exposure [104–107], as appeared to be the case in the WHI. As a result, the observation of suddenly occurring dementias, without its precursor event of mild cognitive impairment (MCI), suggests that these women suffered from vascular events. This is consistent with the well-known effects of orally administered estrogens on liver induction of prothrombotic enzymes [108–110] and the study's observations of an increase in events related to clots, such as strokes, heart attacks and deep venous thromboses [101, 102]. An alternative to oral feminizing estrogens and progestins are nonfeminizing analogues of estrogens and better progestins that likely will avoid first-pass prothrombin induction and plague destabilization.

The WHI studies were not designed to determine if early exposure to estrogens, beginning at the time of the menopause, could affect cognitive decline or the prevalence of AD. Further studies to assess early intervention, as well as other estrogen and progestin preparation and other routes of administration, are clearly needed, particularly in view of the plethora of evidence from animal and clinical studies for the beneficial effects of estrogens on stroke and AD.

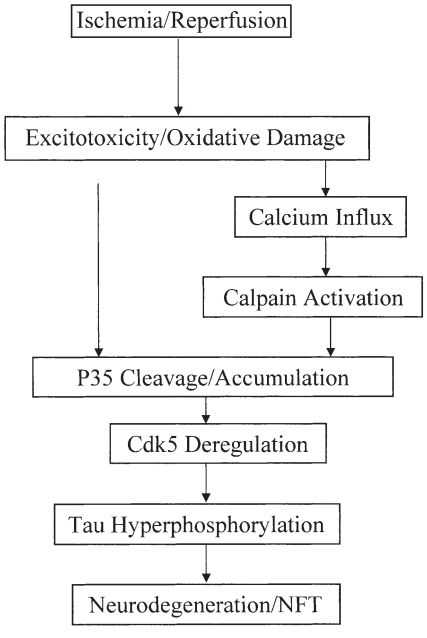

Figure 1. Schematic depiction of the relationship between neurotoxic events and the formation of NFTs.

Conclusions

Estrogens are potent, efficacious compounds in protecting the brain from the damaging effects of stroke and the neuropathology of AD, including neuronal death, AβPP expression, AβPP processing to Aβ, hyperphosphorylation of tau and aberrant neuronal mitosis. Additionally, progestins appear to signal through neuroprotective pathways and exert neuroprotection in a number of experimental models. As such, additional research on these multifaceted compounds is warranted. This additional research should follow three tracts. First, extensive study is needed to determine the cellular targets and molecular events affected by estrogens and progestins to achieve their neuroprotective/anti-AD pathology activities. Sec-

ond, applied animal studies are needed to determine the optimal estrogens and progestins, their formulation and route of administration, dosing schedule and optimal dosing time and duration relative to an acute ischemic event or to the stage of development of AD neuropathology. Third, clinical studies of appropriate estrogen and progestin preparation for efficacy in stroke neuroprotection and AD are warranted, based upon the wealth of in vitro and animal data supporting the efficacy of estrogens and progestins for these conditions.

Acknowledgements. Supported by NIH grants AG10485 (JWS), AG22550 (JWS), AG023330 (MS) and a Young Investigator Award from National Alliance for Schizophrenia and Depression (MS).

1 Thorvaldsen P., Asplund K., Kuulasmaa K., Rajakangas A. M. and Schroll M. (1995) Stroke incidence, case fatality and mortality in the WHO MONICA project. World Health Organization Monitoring Trends and Determinants in Cardiovascular Disease Stroke **26:** 361–367
2 Stegmayr B., Asplund K., Kuulasmaa K., Rajakangas A. M., Thorvaldsen P. and Tuomilehto J. (1997) Stroke incidence and mortality correlated to stroke risk factors in the WHO MONICA Project. An ecological study of 18 populations. Stroke **28:** 1367–1374
3 Clark W. M., Albers G. W., Madden K. P. and Hamilton S. (2000) The rtPA (alteplase) 0- to 6-h acute stroke trial, part A (A0276g): results of a double-blind, placebo-controlled, multicenter study. Thrombolytic therapy in acute ischemic stroke study investigators. Stroke **31:** 811–816
4 Hacke W., Brott T., Caplan L. et al. (1999) Thrombolysis in acute ischemic stroke: controlled trials and clinical experience. Neurology **53:** S3–14
5 Fisher M. and Albers G. W. (1999) Applications of diffusion-perfusion magnetic resonance imaging in acute ischemic stroke. Neurology **52:** 1750–1756
6 Ernst R. L. and Hay J. W. (1994) The US economic and social costs of Alzheimer's disease revisited. Am. J. Public. Health **84:** 1261–1264
7 Murad FaH R. C. Jr. (1985) Estrogens and progestins. In: The Pharmacological Basis of Therapeutics, 7th edn, Gilman A. G., Goodman L. S., Rall T. W. and Murad F. (eds), Macmillan, Company, New York, N. Y. pp. 1414–1439
8 Bishop J. and Simpkins J. W. (1994) Estradiol treatment increases viability of glioma and neuroblastoma cells in vitro. Mol. Cell. Neurosci. **5:** 303–308
9 Simpkins J. W., Rajakumar G., Zhang Y. Q. et al. (1997) Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat. J. Neurosurg. **87:** 724–730
10 Green P. S. and Simpkins J. W. (2000) Neuroprotective effects of estrogens: potential mechanisms of action. Int. J. Dev. Neurosci. **18:** 347–358
11 Behl C., Skutella T., Lezoualc'h F. et al. (1997) Neuroprotection against oxidative stress by estrogens: structure-activity relationship. Mol. Pharmacol. **51:** 535–541
12 Weaver C. E. Jr, Park-Chung M., Gibbs T. T. and Farb D. H. (1997) 17beta-Estradiol protects against NMDA-induced excitotoxicity by direct inhibition of NMDA receptors. Brain Res. **761:** 338–341
13 Singh M., Setalo G. Jr, Guan X., Frail D. E. and Toran-Allerand C. D. (2000) Estrogen-induced activation of the mitogen-activated protein kinase cascade in the cerebral cortex of estrogen receptor-alpha knock-out mice. J. Neurosci. **20:** 1694–1700
14 Honda K., Sawada H., Kihara T. et al. (2000) Phosphatidylinositol 3-kinase mediates neuroprotection by estrogen in cultured cortical neurons. J. Neurosci. Res. **60:** 321–327
15 Wang J., Green P. S. and Simpkins J. W. (2001) Estradiol protects against ATP depletion, mitochondrial membrane potential decline and the generation of reactive oxygen species induced by 3-nitroproprionic acid in SK-N-SH human neuroblastoma cells. J. Neurochem. **77:** 804–811
16 Nilsen J. and Diaz Brinton R. (2003) Mechanism of estrogen-mediated neuroprotection: regulation of mitochondrial calcium and Bcl-2 expression. Proc. Natl. Acad. Sci. USA **100:** 2842–2847
17 Yang S. H., Liu R., Stanley M., Stevens S. M. Jr, Valencia T., Wen Y. et al. (2004) Mitochondrial localization of estrogen receptor beta. Proc. Natl. Acad. Sci. USA **101:** 4130–4135
18 Cammarata P. R., Chu S., Moor A., Yang S.-H. and Simpkins J. W. (2004) Subcellular distribution of native estrogen receptors alpha and beta in cultured human lens epithelial cells. Investigational Eye Res. **78:** 861–871
19 Alkayed N. J., Harukuni I., Kimes A. S., London E. D., Traystman R. J., Hurn P. D. (1998) Gender-linked brain injury in experimental stroke. Stroke **29:** 159–165; discussion 166
20 Dubal D. B., Kashon M. L., Pettigrew L. C. et al. (1998) Estradiol protects against ischemic injury. J. Cereb. Blood Flow Metab. **18:** 1253–1258
21 Sudo S., Wen T. C., Desaki J. et al. (1997) Beta-estradiol protects hippocampal CA1 neurons against transient forebrain ischemia in gerbil. Neurosci. Res. **29:** 345–354
22 Fukuda K., Yao H., Ibayashi S. et al. (2000) Ovariectomy exacerbates and estrogen replacement attenuates photothrombotic focal ischemic brain injury in rats. Stroke **31:** 155–160
23 Mendelowitsch A., Ritz M. F., Ros J., Langemann H. and Gratzl O. (2001) 17beta-Estradiol reduces cortical lesion size in the glutamate excitotoxicity model by enhancing extracellular lactate: a new neuroprotective pathway. Brain Res. **901:** 230–236
24 Chen J., Xu W. and Jiang H. (2001) 17 beta-estradiol protects neurons from ischemic damage and attenuates accumulation of extracellular excitatory amino acids. Anesth. Analg. **92:** 1520–1523
25 Culmsee C., Vedder H., Ravati A. et al. (1999) Neuroprotection by estrogens in a mouse model of focal cerebral ischemia and in cultured neurons: evidence for a receptor-independent antioxidative mechanism. J. Cereb. Blood Flow Metab. **19:** 1263–1269
26 Wise P. M., Dubal D. B., Wilson M. E., Rau S. W., Bottner M. and Rosewell K. L. (2001) Estradiol is a protective factor in the adult and aging brain: understanding of mechanisms derived from in vivo and in vitro studies. Brain Res. Brain Res. Rev. **37:** 313–319
27 Toung T. K., Hurn P. D., Traystman R. J. and Sieber F. E. (2000) Estrogen decreases infarct size after temporary focal ischemia in a genetic model of type 1 diabetes mellitus. Stroke **31:** 2701–2706
28 Carswell H. V., Dominiczak A. F. and Macrae I. M. (2000) Estrogen status affects sensitivity to focal cerebral ischemia in stroke-prone spontaneously hypertensive rats. Am. J. Physiol. Heart Circ. Physiol. **278:** H290–H294
29 Yang S. H., He Z., Wu S. S. et al. (2001) 17-beta estradiol can reduce secondary ischemic damage and mortality of subarachnoid hemorrhage. J. Cereb. Blood Flow Metab. **21:** 174–181
30 Hawk T., Zhang Y. Q., Rajakumar G., Day A. L. and Simpkins J. W. (1998) Testosterone increases and estradiol decreases middle cerebral artery occlusion lesion size in male rats. Brain Res. **796:** 296–298
31 Toung T. J., Traystman R. J. and Hurn P. D. (1998) Estrogen-mediated neuroprotection after experimental stroke in male rats. Stroke **29:** 1666–1670

32 McCullough L. D., Alkayed N. J., Traystman R. J., Williams M. J. and Hurn P. D. (2001) Postischemic estrogen reduces hypoperfusion and secondary ischemia after experimental stroke. Stroke **32:** 796–802

33 Yang S. H., Shi J., Day A. L. and Simpkins J. W. (2000) Estradiol exerts neuroprotective effects when administered after ischemic insult. Stroke **31:** 745–749; discussion 749–750

34 Yang S. H., Liu R., Wu S. S. and Simpkins J. W. (2004) The Use of Estrogens and Related Compounds in the Treatment of Damage from Cerebral Ischemia. In Steroids and the Nervous System. Ann. N. Y. Acad. Sci. **1007:** 101–108

35 De Keyser J., Sulter G. and Luiten P. G. (1999) Clinical trials with neuroprotective drugs in acute ischaemic stroke: are we doing the right thing? Trends Neurosci. **22:** 535–540

36 Falkenstein E., Tillmann H. C., Christ M., Feuring M. and Wehling M. (2000) Multiple actions of steroid hormones – a focus on rapid, nongenomic effects. Pharmacol. Rev. **52:** 513–556

37 Wang X., Simpkins J. W., Dykens J. A. and Cammarata P. R. (2003) Oxidative damage to human lens epithelial cells in culture: estrogen protection of mitochondrial potential, ATP and cell viability. Invest. Ophthalmol. Vis. Sci. **44:** 2067–2075

38 Green P. S., Gordon K. and Simpkins J. W. (1997) Phenolic A ring requirement for the neuroprotective effects of steroids. J. Steroid Biochem. Mol. Biol. **63:** 229–235

39 Moosmann B. and Behl C. (1999) The antioxidant neuroprotective effects of estrogens and phenolic compounds are independent from their estrogenic properties. Proc. Natl. Acad. Sci. USA **96:** 8867–8872

40 Green P. S., Yang S. H., Nilsson K. R., Kumar A. S., Covey D. F. and Simpkins J. W. (2001) The nonfeminizing enantiomer of 17beta-estradiol exerts protective effects in neuronal cultures and a rat model of cerebral ischemia. Endocrinology **142:** 400–406

41 Curtis Hewitt S., Couse J. F. and Korach K. S. (2000) Estrogen receptor transcription and transactivation: estrogen receptor knockout mice: what their phenotypes reveal about mechanisms of estrogen action. Breast Cancer Res. **2:** 345–352

42 Couse J. F. and Korach K. S. (1999) Estrogen receptor null mice: what have we learned and where will they lead us? Endocr. Rev. **20:** 358–417

43 Mehta S. H., Dhandapani K. M., De Sevilla L. M., Webb R. C., Mahesh V. B. and Brann D. W. (2003) Tamoxifen, a selective estrogen receptor modulator, reduces ischemic damage caused by middle cerebral artery occlusion in the ovariectomized female rat. Neuroendocrinology **77:** 44–50

44 Osuka K., Feustel P. J., Mongin A. A., Tranmer B. I. and Kimelberg H. K. (2001) Tamoxifen inhibits nitrotyrosine formation after reversible middle cerebral artery occlusion in the rat. J. Neurochem. **76:** 1842–1850

45 Rossberg M. I., Murphy S. J., Traystman R. J. and Hurn P. D. (2000) LY353381.HCl, a selective estrogen receptor modulator, and experimental stroke. Stroke **31:** 3041–3046

46 Liu R., Yang S. H., Perez E. et al. (2002) Neuroprotective effects of a novel non-receptor-binding estrogen analogue: in vitro and in vivo analysis. Stroke 33: 2485–2491

47 Lee J. M., Grabb M. C., Zipfel G. J. and Choi D. W. (2000) Brain tissue responses to ischemia. J. Clin. Invest. **106:** 723–731.

48 Chrousos G. P., Zoumakis E. and Gravanis A. (2001) The gonadal hormones and Inhibitors. In: Basic and Clinical Pharmacology, 8th edn. Katzung B. G. (ed). McGraw-Hill, New York pp. 679–710

49 Conneely O. M. and Lydon J. P. (2000) Progesterone receptors in reproduction: functional impact of the A and B isoforms. Steroids **65:** 571–577

50 Vegeto E., Shahbaz M. M., Wen D. X., Goldman M. E., O'Malley B. W. and McDonnell D. P. (1992) Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function. Mol. Endocrinol. **7:** 1244–1255

51 Bikle D. D., Halloran B. P., Harris S. T. and Portale A. A. (1992) Progestin antagonism of estrogen stimulated 1,25-dihydroxyvitamin D levels. J. Clin. Endocrinol. Metab. **75:** 519–523

52 Migliaccio A., Piccolo D., Castoria G., Di Domenico M., Bilancio A., Lombardi M. et al. (1998) Activation of the Src/p21ras/Erk pathway by progesterone receptor via cross-talk with estrogen receptor. EMBO J. **17:** 2008–2018

53 Collado M. L., Rodriguez-Manzo G. and Cruz M. L. (1985) Effect of progesterone upon adenylate cyclase activity and cAMP levels on brain areas. Pharmacol. Biochem. Behav. **23:** 501–504

54 Singh M. (2001) Ovarian hormones elicit phosphorylation of Akt and extracellular-signal regulated kinase in explants of the cerebral cortex. Endocrine **14:** 407–415

55 Ke F. C. and Ramirez V. D. (1990) Binding of progesterone to nerve cell membranes of rat brain using progesterone conjugated to 125I-bovine serum albumin as a ligand. J. Neurochem. **54:** 467–472

56 Towle A. C. and Sze P. Y. (1983) Steroid binding to synaptic plasma membrane: differential binding of glucocorticoids and gonadal steroids. J. Steroid. Biochem. **18:** 35–43

57 Zhu Y., Bond J. and Thomas P. (2003) Identification, classification and partial characterization of genes in humans and other vertebrates homologous to a fish membrane progestin receptor. Proc. Natl. Acad. Sci. USA **100:** 2237–2242

58 Zhu Y., Rice C. D., Pang Y., Pace M. and Thomas P. (2003) Cloning, expression and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. Proc. Natl. Acad. Sci. USA **100:** 2231–2236

59 Deutsch S. I., Mastropaolo J. and Hitri A. (1992) GABA-active steroids: endogenous modulators of GABA-gated chloride ion conductance. Clin. Neuropharmacol. **15:** 352–364

60 Lobo R. (1995) Benefits and risks of estrogen replacement therapy. Am. J. Obstet. Gynecol. **173:** 982–989

61 Goodman Y., Bruce A. J., Cheng B. and Mattson M. P. (1996) Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury and amyloid beta-peptide toxicity in hippocampal neurons. J. Neurochem. **66:** 1836–1844

62 Nilson J. and Brinton R. D. (2002) Impact of progestins on estrogen-induced neuroprotection: synergy by progesterone and 19-norprogesterone and antagonism by medroxyprogesterone acetate. Endocrinology **143:** 205–212

63 Nilsen J. and Brinton R. D. (2003) Divergent impact of progesterone and medroxyprogesterone acetate (Provera) on nuclear mitogen-activated protein kinase signaling. Proc. Natl. Acad. Sci. USA 100: 10506–10511

64 Nilson J. and Brinton R. D. (2002) Impact of progestins on estradiol potentiation of the glutamate calcium response. Neuroreport **13:** 825–830

65 Luine V. N. (1985) Estradiol increases choline acetyltransferase activity in specific basal forebrain nuclei and projection areas of female rats. Exp. Neurol. **89:** 484–490

66 Luine V. N., Richards S. T., Wu V. Y. and Beck K. D. (1998) Estradiol enhances learning and memory in a spatial memory task and effects levels of monoaminergic neurotransmitters. Horm. Behav. **34:** 149–162

67 Singh M., Meyer E. M., Millard W. J. and Simpkins J. W. (1994) Ovarian steroid deprivation results in a reversible learning impairment and compromised cholinergic function in female Sprague-Dawley rats. Brain Res. **644:** 305–312

68 Singh M., Meyer E. M. and Simpkins J. W. (1995) The effect of ovariectomy and estradiol replacement on brain-derived neurotrophic factor messenger ribonucleic acid expression in cortical and hippocampal brain regions of female Sprague-Dawley rats. Endocrinology 136: 2320–2324

69 Asbury E. T., Fritts M. E., Horton J. E. and Isaac W. L. (1998) Progesterone facilitates the acquisition of avoidance learning and protects against subcortical neuronal death following prefrontal cortex ablation in the rat. Behav. Brain Res. **97:** 99–106

70 Roof R. L., Duvdevani R., Braswell L. and Stein D. G. (1994) Progesterone facilitates cognitive recovery and reduces secondary neuronal loss caused by cortical contusion injury in male rats. Exp. Neurol. **129:** 64–69

71 Cervantes M., Gonzalez-Vidal M. D., Ruelas R., Escobar A. and Morali 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

72 Gonzalez Deniselle M. C., Lopez Costa J. J., Gonzalez S. L., Labombarda F., Garay L., Guennoun R. et al. (2002) Basis of progesterone protection in spinal cord neurodegeneration. J. Steroid Biochem. Mol. Biol. **83:** 199–209

73 Hoffman G. E., Moore N., Fiskum G. and Murphy A. Z. (2003) Ovarian steroid modulation of seizure severity and hippocampal cell death after kainic acid treatment. Exp. Neurol. **182:** 124–134

74 Roof R. L., Hoffman S. W. and Stein D. G. (1997) Progesterone protects against lipid peroxidation following traumatic brain injury in rats. Mol. Chem. Neuropathol. **31:** 1–11

75 Tatemichi T. K., Desmond D. W., Mayeux R. et al. (1992) Dementia after stroke: baseline frequency, risks, and clinical features in a hospitalized cohort. Neurology **42:** 1185–1193

76 Henon H., Durieu I., Lucas C., Godefroy O., Pasquier F. and Leys D. (1996) Dementia in stroke. Neurology **47:** 852–853

77 Loeb C., Gandolfo C., Croce R. and Conti M. (1992) Dementia associated with lacunar infarction. Stroke **23:** 1225–1229

78 Pasquier F. and Leys D. (1997) Why are stroke patients prone to develop dementia? J. Neurol. **244:** 135–142

79 Tatemichi T. K., Paik M., Bagiella E. et al. (1994) Risk of dementia after stroke in a hospitalized cohort: results of a longitudinal study. Neurology **44:** 1885–1891

80 Rocca W. A., Bonaiuto S., Lippi A. et al. (1990) Prevalence of clinically diagnosed Alzheimer's disease and other dementing disorders: a door-to-door survey in Appignano, Macerata Province, Italy. Neurology **40:** 626–631

81 Frisoni G. B., Geroldi C., Bianchetti A. et al. (1994) Apolipoprotein E epsilon 4 allele frequency in vascular dementia and Alzheimer's disease. Stroke **25:** 1703–1704

82 Lenzen H. J., Assmann G., Buchwalsky R. and Schulte H. (1986) Association of apolipoprotein E polymorphism, low-density lipoprotein cholesterol and coronary artery disease. Clin. Chem. **32:** 778–781

83 Shimano H., Ishibashi S., Murase T. et al. (1989) Plasma apolipoproteins in patients with multi-infarct dementia. Atherosclerosis **79:** 257–260

84 Pedro-Botet J., Senti M., Nogues X. et al. (1992) Lipoprotein and apolipoprotein profile in men with ischemic stroke. Role of lipoprotein(a), triglyceride-rich lipoproteins, and apolipoprotein E polymorphism. Stroke **23:** 1556–1562

85 Saunders A. M. and Roses A. D. (1993) Apolipoprotein E4 allele frequency, ischemic cerebrovascular disease and Alzheimer's disease. Stroke **24:** 1416–1417

86 Stephenson D. T., Rash K. and Clemens J. A. (1992) Amyloid precursor protein accumulates in regions of neurodegeneration following focal cerebral ischemia in the rat. Brain Res. **593:** 128–135

87 Shi J., Yang S. H., Stubley L., Day A. L. and Simpkins J. W. (2000) Hypoperfusion induces overexpression of beta-amyloid precursor protein mRNA in a focal ischemic rodent model. Brain Res. **853:** 1–4

88 Yoshimura M., Yamanouchi H., Kuzuhara S. et al. (1992) Dementia in cerebral amyloid angiopathy: a clinicopathological study. J. Neurol. **239:** 441–450

89 Ince P. G., McArthur F. K., Bjertness E., Torvik A., Candy J. M. and Edwardson J. A. (1995) Neuropathological diagnoses in elderly patients in Oslo: Alzheimer's disease, Lewy body disease, vascular lesions. Dementia **6:** 162–168

90 Ikeda K., Akiyama H., Arai T. et al. (2000) Neurons containing Alz-50-immunoreactive granules around the cerebral infarction: evidence for the lysosomal degradation of altered tau in human brain? Neurosci. Lett. **284:** 187–189

91 Guenette S. Y. and Tanzi R. E. (1999) Progress toward valid transgenic mouse models for Alzheimer's disease. Neurobiol. Aging **20:** 201–211

92 Sathasivam K., Hobbs C., Mangiarini L. et al. (1999) Transgenic models of Huntington's disease. Philos. Trans. R. Soc. Lond. B. Biol. Sci. **354:** 963–969

93 Borchelt D. R., Wong P. C., Sisodia S. S. and Price D. L. (1998) Transgenic mouse models of Alzheimer's disease and amyotrophic lateral sclerosis. Brain Pathol. **8:** 735–757

94 Vila M. and Przedborski S. (2003) Targeting programmed cell death in neurodegenerative diseases. Nat. Rev. Neurosci. **4:** 365–735

95 Mattson M. P. (2000) Apoptosis in neurodegenerative disorders. Nat. Rev. Mol. Cell. Biol. **1:** 120–129

96 Graham S. H. and Chen J. (2001) Programmed cell death in cerebral ischemia. J. Cereb. Blood Flow Metab. **21:** 99–109

97 Wen Y., Yang S., Liu R., Brun-Zinkernagel A. M., Koulen P. and Simpkins J. W. (2004) Transient cerebral ischemia induces aberrant neuronal cell cycle reentry and Alzheimer's disease-like tauopathy in female rats. J. Biol. Chem. **279:** 22684–22692

98 Tseng H. C., Zhou Y., Shen Y. and Tsai L. H. (2002) A survey of Cdk5 activator p35 and p25 levels in Alzheimer's disease brains. FEBS Lett. **523:** 58–62

99 Patzke H. and Tsai L. H. (2002) Calpain-mediated cleavage of the cyclin-dependent kinase-5 activator p39 to p29. J. Biol. Chem. **277:** 8054–8060

100 Lee M. S. and Tsai L. H. (2003) Cdk5: One of the links between senile plaques and neurofibrillary tangles? J. Alzheimers Dis. **5:** 127–137

101 Shumaker S. A., Legault C., Rapp S. R., Thal L., Wallace R. B., Ockene J. K. et al. (2003) Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. JAMA **289:** 2651–2662

102 Rapp S. R., Espeland M. A., Shumaker S. A., Henderson V. W., Brunner R. L., Manson J. E. et al. (2003) Effect of estrogen plus progestin on global cognitive function in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. JAMA **289:** 2663–2672

103 Raggi P., Callister T. Q., Cooil B., He Z. X., Lippolis N. J., Russo D. J. et al. (2000) Identification of patients at increased risk of first unheralded acute myocardial infarction by electron-beam computed tomography. Circulation **101:** 850–855

104 Zegura B., Keber I., Sebestjen M. and Koenig W. (2003) Related Articles, Double blind, randomized study of estradiol replacement therapy on markers of inflammation, coagulation and fibrinolysis. Atherosclerosis **168:** 123–129

105 Wingrove C. S., Garr E., Godsland I. F. and Stevenson J. C. (1998) Related Articles, 17beta-oestradiol enhances release of matrix metalloproteinase-2 from human vascular smooth muscle cells. Biochim. Biophys. Acta **1406:** 169–174

106 Zanger D., Yang B. K., Ardans J., Waclawiw M. A., Csako G., Wahl L. M. et al. (2000) Related Articles, Divergent effects of hormone therapy on serum markers of inflammation in postmenopausal women with coronary artery disease on appropriate medical management. J. Am. Coll. Cardiol. **36:** 1797–1802

107 Ikeda U. and Shimada K. (2003) Related Articles, Matrix metalloproteinases and coronary artery diseases. Clin. Cardiol. **26:** 55–59

108 De Lignieres B., Basdevant A., Thomas G., Thalabard J. C., Mercier-Bodard C., Conard J. et al. (1986) Related Articles, Biological effects of estradiol-17 beta in postmenopausal women: oral versus percutaneous administration. J. Clin. Endocrinol. Metab. **62:** 536–551

109 Scarabin P. Y., Alhenc-Gelas M., Plu-Bureau G., Taisne P., Agher R. and Aiach M. (1997) Related Articles, Effects of oral and transdermal estrogen/progesterone regimens on blood coagulation and fibrinolysis in postmenopausal women. A randomized controlled trial. Arterioscler. Thromb. Vasc. Biol. **17:** 3071–3078

110 Alkjaersig N., Fletcher A. P., de Ziegler D., Steingold K. A., Meldrum D. R. and Judd H. L. (1988) Related Articles, Blood coagulation in postmenopausal women given estrogen treatment: comparison of transdermal and oral administration. J. Lab. Clin. Med. **111:** 224–228