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17β-Estradiol Attenuates Oxidative Impairment of Synaptic Na^+/K^+-ATPase Activity, Glucose Transport, and Glutamate Transport Induced by Amyloid β-Peptide and Iron

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Synapse loss, deposits of amyloid β-peptide (Aβ), impaired energy metabolism, and cognitive deficits are defining features of Alzheimer's disease (AD). Estrogen replacement therapy reduces the risk of developing AD in postmenopausal women. Because synapses are likely sites for initiation of neurodegenerative cascades in AD, we tested the hypothesis that estrogens act directly on synapses to suppress oxidative impairment of membrane transport systems. Exposure of rat cortical synaptosomes to Aβ25-35 (Aβ) and $FeSO_4$ induced membrane lipid peroxidation and impaired the function of the plasma membrane Na^+/K^+-ATPase, glutamate transporter, and glucose transporter. Pretreatment of synaptosomes with 17β-estradiol or estriol largely prevented impairment of Na^+/K^+-ATPase activity, glutamate transport, and glucose transport; other steroids were relatively ineffective. 17β-Estradiol suppressed membrane lipid peroxidation induced by Aβ and $FeSO_4$, but did not prevent impairment of membrane transport systems by 4-hydroxynonenal (a toxic lipid peroxidation product), suggesting that an antioxidant property of 17β-estradiol was responsible for its protective effects. By suppressing membrane lipid peroxidation in synaptic membranes, estrogens may prevent impairment of transport systems that maintain ion homeostasis and energy metabolism, and thereby forestall excitotoxic synaptic degeneration and neuronal loss in disorders such as AD and ischemic stroke. J. Neurosci. Res. 50:522–530, 1997. © 1997 Wiley-Liss, Inc.

Key words: Alzheimer's disease; estrogen; free radicals; glucocorticoids; hydroxynonenal; lipid peroxidation; mitochondrial electron transport; neuronal death; progesterone; thiobarbituric acid

INTRODUCTION

Alzheimer's disease (AD) is characterized clinically by age-related progressive cognitive deficits and histopathologically by accumulations of senile plaques comprised largely of amyloid β-peptide (Aβ) and neurofibrillary tangles which consist of massive accumulations of intraneuronal filaments comprised largely of the microtubule-associated protein tau (for review see Selkoe, 1989). Additional prominent features of AD, which are highly correlated with cognitive decline, are synapse loss (DeKosky and Scheff, 1990; Terry et al., 1991) and impaired glucose transport (Kalaria and Harik, 1989; Hoyer, 1991; Jagust et al., 1991; Sims, 1990). Reduced glucose uptake may occur early in the disease process prior to neuronal degeneration (Pettegrew et al., 1994; Kennedy et al., 1995; Reiman et al., 1996). Decreases in the activities of pyruvate dehydrogenase and ketoglutarate dehydrogenase complexes further suggest the presence of a profound deficit in mitochondrial metabolism in AD (Blass, 1993). The mechanisms underlying the metabolic alterations and neurodegenerative processes in AD are not understood, but increasing data implicate oxidative stress and formation of fibrillar deposits of Aβ (for review see Mattson et al., 1996).

Increased levels of protein (Smith et al., 1991), lipid (Lovell et al., 1995), and DNA (Moccoci et al., 1994) oxidation have been documented in postmortem tissue taken from vulnerable regions of AD brain. Moreover, the

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major proteins comprising neurofibrillary tangles (tau) and senile plaques (Aβ) are glycated (Smith et al., 1994; Vitek et al., 1994; Yan et al., 1995), suggesting high levels of oxidative stress in the microenvironment of degenerating neurons. Strong links between Aβ deposition and neurodegeneration in AD have been made based upon molecular, genetic, histopathological, and experimental data (for review see Mattson et al., 1996; Hardy, 1997). Aβ induces membrane lipid peroxidation and disrupts ion homeostasis in cultured neurons and synaptosomes (Mattson et al., 1992; Behl et al., 1994; Butterfield et al., 1994; Goodman and Mattson, 1994; Goodman et al., 1994; Mark et al., 1995). Aβ and lipid peroxidation can impair the function of several critical membrane proteins including ion-motive ATPases (Mark et al., 1995, 1997a), glutamate transporters (Harris et al., 1995; Keller et al., 1997), glucose transporters (Mark et al., 1997b), and GTP-binding proteins (Kelly et al., 1996; Blanc et al., 1997). 4-Hydroxynonenal (HNE), an aldehydic product of lipid peroxidation, may mediate the damaging effects of Aβ on cells by conjugating directly to membrane proteins and impairing their function (Keller et al., 1997; Mark et al., 1997a,b).

Postmenopausal women who take estrogen replacement therapy have a reduced risk for developing cardiovascular disease (Schwartz et al., 1995) and certain forms of cancer (Vogel, 1996), and have an increased lifespan (Ettinger et al., 1996). Recent studies have shown that estrogens reduce the risk of developing AD (Henderson et al., 1994; Tang et al., 1996) and retard the decline in cognitive performance that occurs during ''normal'' aging (Sherwin, 1994). The mechanisms whereby estrogens prevent or delay age-related neuronal degeneration are unclear. We recently reported that 17β-estradiol (Est) and estriol can protect cultured hippocampal neurons from being damaged and killed by oxidative insults, including exposure to Aβ (Goodman et al., 1996). The estrogens suppressed membrane lipid peroxidation and stabilized calcium homeostasis in neurons exposed to $FeSO_4$ and Aβ, effects that were apparently due to inherent antioxidant properties of the steroids. In the present study, we tested the hypothesis that estrogens can act directly on synapses to preserve their function in the face of potentially toxic exposures to Aβ and other insults that promote membrane lipid peroxidation.

MATERIALS AND METHODS

Materials

Aβ25-35 was purchased from Bachem (Torrance, CA); the peptide was stored lyophilized, and a 1 mM stock (in sterile water) was prepared 2 hr prior to experiments. HNE (Cayman Chemical Company, Ann Arbor, MI) was prepared as a ×500 stock in ethanol; an equivalent amount of ethanol vehicle (0.2% final concentration) was added to control synaptosomal preparations. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Fiske-Subbarao reagent, $FeSO_4$, and 2-thiobarbituric acid were from Sigma (St. Louis, MO). Steroids (Sigma) were prepared as 500× stocks in ethanol. [^{3}H]-glutamate was from Amersham (Arlington Heights, IL) and [^{3}H]-2-deoxy-glucose was from New England Nuclear (Boston, MA, Sigma).

Preparation and Experimental Treatment of Synaptosomes

These methods were similar to those described previously (Keller et al., 1997). Briefly, cerebral hemispheres from adult female Sprague-Dawley rats (250–300 g) were cut into small fragments and homogenized in a solution containing 0.32 M sucrose, 4 μg/ml pepstatin, 5 μg/ml aprotinin, 20 μg/ml trypsin inhibitor, 4 μg/ml leupeptin, 0.2 mM PMSF, 2 mM EDTA, 2 mM EGTA, and 20 mM Hepes. The homogenate was centrifuged for 10 min at 1,500 rpm (4°C), and the supernatant was then centrifuged for 10 min at 13,000 rpm (4°C). The pellet was resuspended in 1.5 ml of 0.32 M sucrose and placed on top of a sucrose gradient (7 ml 1.18 M sucrose, pH 8.5; 7 ml 1 M sucrose, pH 8.0; 7 ml 0.85 M sucrose, pH 8), and centrifuged at 22,000 rpm for 2 hr at 4°C. Synaptosomes in the 1 M sucrose/1.18 M sucrose interface were removed, resuspended in phosphate buffered saline (PBS), and centrifuged at 10,000 rpm for 10 min (4°C). The purity of synaptosomes prepared using similar methods was documented in previous studies (Whittaker et al., 1964). Protein concentrations in synaptosomal preparations were determined (Pierce BCA kit, Rockford, IL) and equivalent amounts of synaptosomes were aliquoted to wells of 96-well plates or 1.5 ml Eppendorf tubes depending upon the assay to be performed. Synaptosomes were incubated in Locke's solution (NaCl, 154 mM; KCl, 5.6 mM; $CaCl_2$, 2.3 mM; $MgCl_2$, 1.0 mM; $NaHCO_3$, 3.6 mM; glucose, 5 mM; Hepes, 5 mM; pH 7.2), and experimental treatments were added by dilution into the synaptosomal suspension.

Analyses of Lipid Peroxidation and Mitochondrial Function

Levels of malondialdehyde, an aldehydic product of lipid peroxidation, were measured as described previously (Korachich and Mishra, 1980; Goodman et al., 1996). Briefly, synaptosomes were placed in Eppendorf tubes (200 μg/tube) in 0.4 ml Locke's buffer and incubated at 37°C in a CO_2 incubator. Following experimental treatment, 0.4 ml of ice-cold 10% trichloroacetic acid (TCA) was added, and the synaptosomes were pelleted by centrifugation. Thiobarbituric acid reactive substances

(TBARS) reagent (0.335% 2-thiobarbituric acid in 50% glacial acidic acid) was added and samples were incubated at 100°C for 30 min. Samples were then cooled, 400 µl of water-saturated butanol was added, and samples were vortexed and centrifuged at 2,000 rpm for 5 min. A 100 µl aliquot of the upper organic phase was removed, and fluorescence was quantified using a fluorescence plate reader (518 nm excitation and 588 nm emission). Values of TBARS fluorescence were expressed on a per microgram protein basis. An assay that quantifies MTT reduction was used as a measure of mitochondrial function (Mosmann, 1983; Shearman et al., 1995). Briefly, following experimental treatment MTT solution (5 mg/ml PBS) was mixed with the synaptosomes (1:10; MTT: synaptosomes, v:v) and allowed to incubate for 30 min. The synaptosomes were pelleted by centrifugation, the pellet was solubilized in dimethylsulfoxide, and absorbance of each sample was quantified using a plate reader.

Na^+/K^+-ATPase Activity Assay

Levels of Na^+/K^+-ATPase activity in synaptosomal membranes were quantified as described in our previous study (Mark et al., 1995). Briefly, reactions were carried out in 96-well microplates containing 2 µg of membrane protein incubated in a solution containing (mM): histidine, 18; imidazole, 18; NaCl, 80; KCl, 15; $MgCl_2$, 3; EGTA, 0.1; pH 7.1 (with or without 0.2 mM ouabain). Following a 10 min preincubation at 37°C, the reaction was started by adding 10 µl of ATP (3 mM final concentration). After 60 min the reaction was stopped by adding 25 µl of 5% sodium dodecyl sulfate (SDS), and levels of inorganic phosphate were quantified using the colorimetric method of Fiske and Subbarow (1925). The plates were read on a Bio-tek EL-340 plate reader at 630 nm, and the absorbance values obtained were converted to activity values by linear regression using a standard curve generated with sodium monobasic phosphate. The Na^+/K^+-ATPase activity was determined by subtracting the value obtained in the samples containing ouabain from the value obtained in samples lacking ouabain.

Glucose and Glutamate Transport Assays

Following treatments, and just prior to the glucose uptake assay, synaptosomes (200 µg/tube) were washed 3 times in glucose-free Locke's solution and the assay was started by the addition of 1.5 µCi of [^{3}H]-2-deoxyglucose. Seven minutes later the assay was stopped by pelleting the synaptosomes, washing twice with glucose-free Locke's solution, and lysing the synaptosomes in 200 µl of a 1% SDS/PBS solution (an aliquot was used for protein determination using a Pierce BCA kit). Radioactivity was counted in a Packard 2500TR liquid scintillation counter and results were expressed as cpm per microgram protein. The rate of [^{3}H]-glutamate uptake into synaptosomes was quantified using methods similar to those described previously (Pogun et al., 1994; Oppong et al., 1995). Following experimental treatments, synaptosomes (200 µg/tube) were incubated for 7 min with [^{3}H]-glutamate (0.1 µCi/ml). The preparations were then washed 3 times in Locke's solution using Whatman filters in a vacuum filtration apparatus. The filters were then placed in scintillation vials containing Scintiverse and radioactivity was determined by scintillation spectroscopy. The rates of glucose and glutamate uptake by synaptosomes remained constant under basal conditions throughout the time course of experiments (up to 4 hr).

RESULTS

17β-Estradiol Attenuates Aβ- and Iron-Induced Impairment of Na^+/K^+-ATPase Activity, Glucose Transport, and Glutamate Transport in Synaptosomes

Our previous studies (Mark et al., 1995; Keller et al., 1997) and preliminary data (not shown) demonstrated that exposure of synaptosomes for 2–4 hr to 50 µM $FeSO_4$ or 50 µM Aβ25-35 (Aβ) consistently resulted in large 50–80% decreases in Na^+/K^+-ATPase activity, glutamate transport, and glucose transport without causing lactate dehydrogenase release (i.e., no lysis) from the synaptosomes. When synaptosomes were pretreated for 60 min with 17β-estradiol (Est) and then exposed to $FeSO_4$ and Aβ, the impairment of Na^+/K^+-ATPase activity was significantly decreased (Fig. 1). Est at a concentration of 10 µM completely prevented impairment of Na^+/K^+-ATPase activity by both $FeSO_4$ and Aβ; 100 nM Est was also effective in preventing impairment of Na^+/K^+-ATPase activity induced by $FeSO_4$, but was less effective in protecting against Aβ. In vehicle-treated control synaptosomes, $FeSO_4$ and Aβ caused 40–50% reductions in glucose transport; pretreatment of synaptosomes with Est abrogated, in a concentration-dependent manner, impairment of glucose transport induced by $FeSO_4$ and Aβ (Fig. 2). $FeSO_4$ and Aβ each induced an approximately 60% reduction in glutamate transport in control synaptosomes; Est at concentrations of 100 nM and 10 µM completely prevented impairment of glutamate transport induced by $FeSO_4$ and Aβ (Fig. 3).

Specificity of the protective effects of Est was assessed by determining whether other steroids modified $FeSO_4$-induced impairment of glucose and glutamate transport. Synaptosomes were pretreated with vehicle, 10 µM Est, 10 µM estriol, 10 µM corticosterone, or 10 µM progesterone and then exposed for 3 hr to 50 µM $FeSO_4$. Est significantly attenuated the $FeSO_4$-induced impairments of glucose and glutamate transport (Table I). Estriol and progesterone significantly reduced the $FeSO_4$-

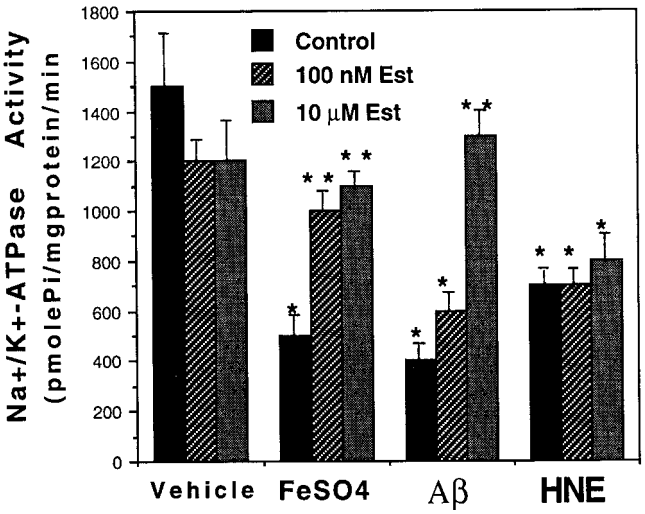

Fig. 1. Est attenuates impairment of Na^+/K^+-ATPase activity induced by $FeSO_4$ and Aβ, but not that induced by HNE, in cortical synaptosomes. Synaptosomes were pretreated for 1 hr with 0.2% ethanol (control) or the indicated concentrations of Est, and were then exposed for 3 hr to vehicle, 50 μM $FeSO_4$, 50 μM Aβ, or 10 μM HNE. Na^+/K^+-ATPase activity was then quantified (see Materials and Methods), and values are the mean ± SEM of determinations made in 4 separate preparations. $*P < 0.01$ compared to value in vehicle-treated control cultures; $**P < 0.01$ compared to corresponding control value (ANOVA with Scheffe's posthoc tests).

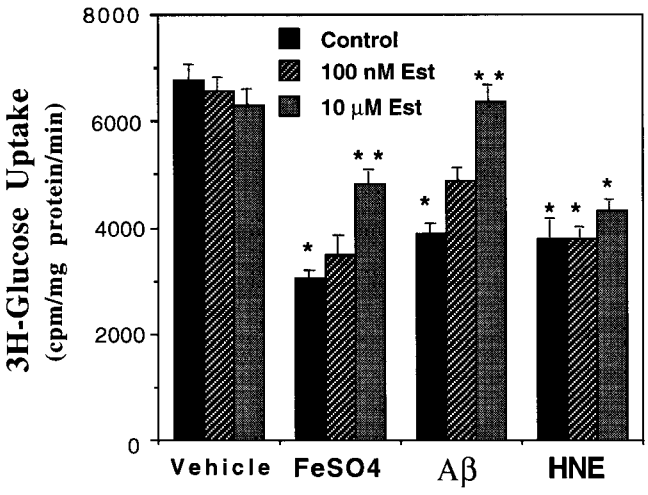

Fig. 2. Est attenuates impairment of glucose transport induced by $FeSO_4$ and Aβ, but not that induced by HNE, in cortical synaptosomes. Synaptosomes were pretreated for 1 hr with 0.2% ethanol (control) or the indicated concentrations of Est, and were then exposed for 3 hr to vehicle, 50 μM $FeSO_4$, 50 μM Aβ, or 10 μM HNE. Glucose transport was then quantified (see Materials and Methods), and values are the mean ± SEM of determinations made in 6 separate preparations. $*P < 0.01$ compared to value in vehicle-treated control cultures; $**P < 0.01$ compared to corresponding control value (ANOVA with Scheffe's posthoc tests).

induced impairment of glucose transport, although to a lesser extent than Est (Table I). In contrast, corticosterone had no significant effect on $FeSO_4$-induced impairment of glucose transport. Est and estradiol significantly attenuated $FeSO_4$-induced decreases in glutamate transport, while progesterone and corticosterone did not attenuate $FeSO_4$-induced impairment of glutamate transport (Table I). Corticosterone significantly enhanced $FeSO_4$-induced impairment of glutamate transport (Table I).

Evidence That the Protective Mechanism of Action of 17β-Estradiol Involves Suppression of Membrane Lipid Peroxidation

$FeSO_4$ and Aβ impair ion-motive ATPases and glucose and glutamate transporters by inducing membrane lipid peroxidation (Volterra et al., 1994; Mark et al., 1995, 1997a,b). We therefore quantified relative levels of membrane lipid peroxidation using the TBARS assay in vehicle- and Est-pretreated synaptosomes exposed to $FeSO_4$ and Aβ. As expected, $FeSO_4$ and Aβ each induced a large increase in TBARS fluorescence during a 3 hr exposure period (Fig. 4). Pretreatment of synaptosomes with Est at concentrations of 0.1–10 μM largely prevented the increase in TBARS fluorescence otherwise induced by $FeSO_4$ and Aβ (Fig. 4). HNE is an aldehydic product of lipid peroxidation which is generated in cultured hippocampal neurons and synaptosomes exposed to $FeSO_4$ and Aβ, wherein it conjugates to membrane transporters and impairs their function (Keller et al., 1997; Mark et al., 1997a,b). Exposure of synaptosomes for 3 hr to 10 μM HNE caused highly significant decreases in Na^+/K^+-ATPase activity (Fig. 1) and glucose (Fig. 2) and glutamate (Fig. 3) transport. Pretreatment of synaptosomes with Est at concentrations of 0.1–10 μM did not modify HNE-induced impairment of Na^+/K^+-ATPase activity, glucose transport, or glutamate transport. Progesterone and corticosterone also did not alter HNE-induced impairment of glucose or glutamate transport (Table I). Because HNE is a downstream effector of lipid peroxidation-induced damage, these findings further support inhibition of lipid peroxidation as the primary mechanism of action of Est.

17β-Estradiol Prevents Impairment of Mitochondrial Function by $FeSO_4$ and Aβ

Exposure of synaptosomes to $FeSO_4$ and Aβ for 3 hr caused significant 35–45% decreases in levels of MTT reduction (Fig. 5). Pretreatment of synaptosomes with Est largely eliminated the decrease in MTT reduction otherwise induced by $FeSO_4$ and Aβ. HNE caused a large decrease in levels of MTT reduction, and this action of

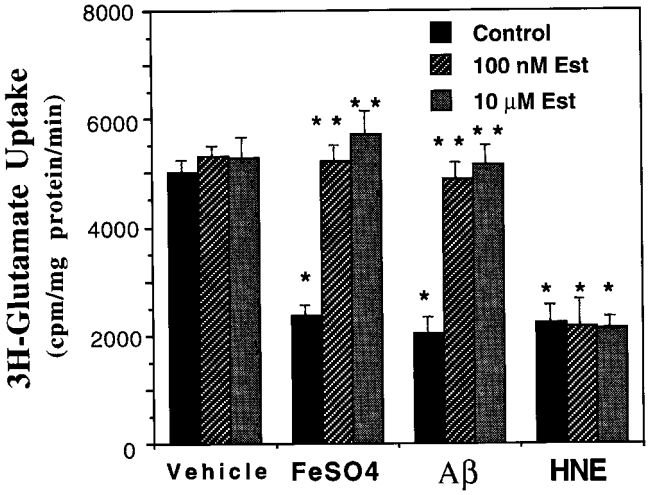

Fig. 3. Est attenuates impairment of glutamate transport induced by $FeSO_4$ and Aβ, but not that induced by HNE, in cortical synaptosomes. Synaptosomes were pretreated for 1 hr with 0.2% ethanol (control) or the indicated concentrations of Est, and were then exposed for 3 hr to vehicle, 50 μM $FeSO_4$, 50 μM Aβ, or 10 μM HNE. Glutamate transport was then quantified (see Materials and Methods), and values are the mean ± SEM of determinations made in 6 separate preparations. *$P < 0.01$ compared to value in vehicle-treated control cultures; **$P < 0.01$ compared to corresponding control value (ANOVA with Scheffe's posthoc tests).

HNE was unaffected by Est pretreatment (Fig. 5). When taken together with the data obtained using TBARS assay (Fig. 4), the results obtained from MTT studies suggest that Est prevents impairment of mitochondrial function by suppressing membrane lipid peroxidation.

DISCUSSION

The present data demonstrate that Est can act directly on synapses and therein protect critical membrane transport systems from oxidative impairment. Pretreatment of synaptosomes with Est and estriol significantly attenuated impairments of Na^+/K^+-ATPase activity, glucose transport, and glutamate transport induced by $FeSO_4$ and Aβ. Progesterone partially attenuated $FeSO_4$-induced impairment of glucose transport, while corticosterone exacerbated $FeSO_4$-induced impairment of glutamate transport. The protective action of Est could not have resulted from transcription-dependent mechanisms mediated by classic cytosolic receptors because the synaptosomal preparations lack nuclei. Indeed, previous studies of cultured neural cells provided evidence that estrogens can directly protect neurons against excitotoxic and oxidative insults, by a mechanism not involving classic cytosolic steroid receptors (Behl et al., 1995; Goodman et al., 1996; Green et al., 1996). Est was effective at concentrations of 0.1–1 μM, which are concentrations similar to those previously shown to have antioxidant activity in various in non-neural systems (Subbiah et al., 1993; Parthasarathy et al., 1994). These concentrations are considerably above circulating levels of such estrogens in postmenopausal women (0.1–1 nM) and slightly above those observed in pregnant women (approximately 50 nM). Suppression of $FeSO_4$- and Aβ-induced lipid peroxidation by Est suggests that antioxidant activity of the steroid is likely responsible for its protective actions, and is consistent with previous data from studies of non-neuronal cells (Subbiah et al., 1993; Keaney et al., 1994; Ruiz-Larrea et al., 1994). However, because there is evidence for the presence of membrane receptors for steroids (including Est) in neurons (Paul and Purdy, 1992; Horvat et al., 1995), the possibility that such receptors mediate the protective actions of Est cannot be ruled out.

Proper functioning of ion-motive ATPases, glutamate transporters, and glucose transporters is perhaps more crucial at synapses than in other regions of neurons. This is because it is at the synapse where glutamate receptors are concentrated and the majority of calcium influx occurs, in both physiological and pathological conditions (Olney et al., 1979; Novelli et al., 1988). Moreover, the concentration of mitochondria in synaptic regions makes them sites of high metabolic activity and accordingly high levels of oxyradical production (LeBel and Bondy, 1992). Impaired function of the Na^+/K^+-ATPase results in membrane depolarization and promotes potentially toxic calcium influx through voltage-dependent calcium channels and N-methyl-D-aspartate (NMDA) receptor channels (Miller et al., 1989; Choi, 1994). Impairment of glutamate transport can lead to excessive activation of glutamate receptors and consequent calcium influx and oxidative stress (Mattson et al., 1995; Rothstein et al., 1996). Impaired glucose transport promotes ATP depletion and thereby compromises a host of energy-dependent processes that occur in synapses. By impairing such critical plasma membrane transport proteins, lipid peroxidation promotes calcium overload and excitotoxic cascades (Zhang et al., 1993; Mark et al., 1997a,b). Indeed, previous studies have shown that oxidative damage to membrane transport systems, induced by Aβ or $FeSO_4$ in cultured neurons, leads to elevations of intracellular calcium levels and apoptotic and/or excitotoxic cell death (Mattson et al., 1992; Mark et al., 1995; Zhou et al., 1996; Kruman et al., 1997). Our data suggest that, by suppressing membrane lipid peroxidation, estrogens preserve function of ion-motive ATPases, and glutamate and glucose transporters. In this way, estrogens may prevent excitotoxic synaptic degeneration. Indeed, it was recently reported that estrogens can protect cultured rat hippocampal neurons against glutamate-induced calcium overload and cell death (Goodman et al., 1996).

TABLE I. Protection Against Oxidative Impairment of Glutamate and Glucose Uptake Is Steroid Specific†

	^{3}H-glucose uptake			^{3}H-glutamate uptake		
Treatment	Vehicle	$FeSO_4$	HNE	Vehicle	$FeSO_4$	HNE
Control	100 ± 9	35 ± 4*	45 ± 3**	100 ± 4	51 ± 7**	49 ± 10**
17β-Estradiol	115 ± 5	86 ± 7*	45 ± 2**	140 ± 10**	79 ± 6*	45 ± 3**
Estriol	100 ± 6	50 ± 6**	48 ± 2**	106 ± 9	73 ± 6**	44 ± 3**
Progesterone	99 ± 5	70 ± 3**	46 ± 3**	144 ± 6**	48 ± 6**	34 ± 3**
Corticosterone	93 ± 6	32 ± 2*	43 ± 3**	98 ± 1	30 ± 5**	22 ± 2**

†Synaptosomes were pretreated for 1 hr with the indicated steroids (10 μM each) and then exposed for 3 hr to vehicle (0.2% ethanol), 50 μM $FeSO_4$, 50 μM Aβ, or 10 μM HNE. Values are expressed as percent control and represent the mean ± SEM of determinations made in 4 preparations.
*$P < 0.05$; **$P < 0.01$ compared to corresponding control value (ANOVA with Scheffe's posthoc tests).

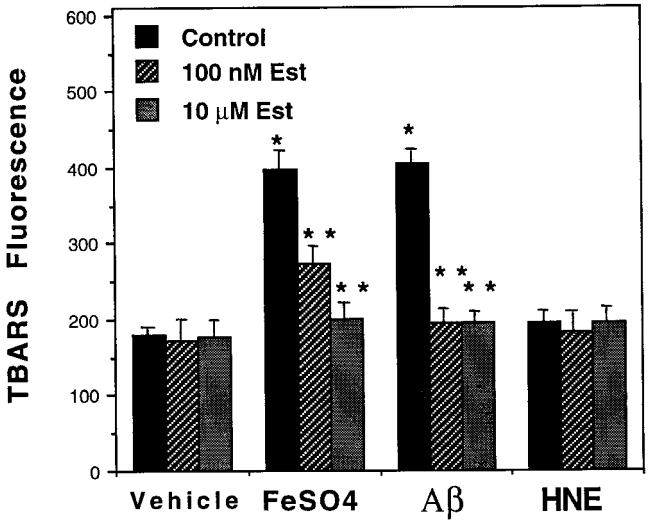

Fig. 4. Est attenuates synaptosomal membrane lipid peroxidation induced by $FeSO_4$ and Aβ. Synaptosomes were pretreated for 1 hr with 0.2% ethanol (control) or the indicated concentrations of Est, and were then exposed for 3 hr to vehicle, 50 μM $FeSO_4$, 50 μM Aβ, or 10 μM HNE. TBARS fluorescence was then quantified (see Materials and Methods), and values are the mean ± SEM of determinations made in 6 separate preparations. *$P < 0.01$ compared to value in vehicle-treated control cultures; **$P < 0.01$ compared to corresponding control value (ANOVA with Scheffe's posthoc tests).

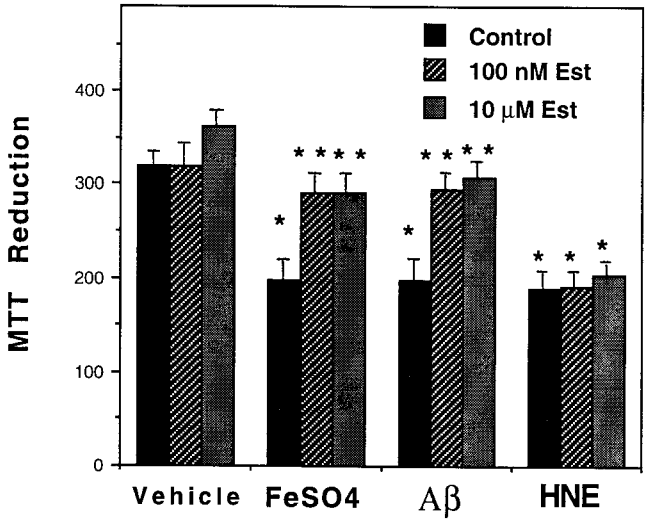

Fig. 5. Est attenuates impairment of mitochondrial function induced by $FeSO_4$ and Aβ, but not that induced by HNE, in cortical synaptosomes. Synaptosomes were pretreated for 1 hr with 0.2% ethanol (control) or the indicated concentrations of Est, and were then exposed for 3 hr to vehicle, 50 μM $FeSO_4$, 50 μM Aβ, or 10 μM HNE. MTT reduction (a measure of mitochondrial function) was then quantified (see Materials and Methods), and values are the mean ± SEM of determinations made in 6 separate preparations. *$P < 0.01$ compared to value in vehicle-treated control cultures; **$P < 0.01$ compared to corresponding control value (ANOVA with Scheffe's posthoc tests).

HNE has recently been shown to be a key mediator of oxidative impairment of neuronal membrane transporters. Exposure of cultured hippocampal neurons to $FeSO_4$ and Aβ results in the production of low micromolar concentrations of HNE (Mark et al., 1997a). Such concentrations of HNE can impair Na^+/K^+-ATPase activity and glucose transport in cultured hippocampal neurons (Mark et al., 1997a,b), and glutamate transport in synaptosomes (Keller et al., 1997). The latter studies provided evidence that HNE covalently binds to glucose and glutamate transport proteins, and may thereby impair their function. In addition, HNE appears to be an important mediator of neuronal apoptosis resulting from exposure to oxidative insults including $FeSO_4$ and Aβ (Kruman et al., 1997). We found that Est was ineffective in protecting synaptosomes against HNE-induced impairment of glucose and glutamate transport. Taken together with the ability of Est to suppress neuronal membrane lipid peroxidation (Goodman et al., 1996), these findings suggest that Est acts mainly as an antioxidant, rather than by preventing events downstream of lipid peroxidation.

The antioxidant properties of estrogen may provide an explanation for its rather remarkable ability to protect

cells in many different organ systems against age-related disease. Free radical-mediated cellular damage is implicated in each of the prominent disorders in which estrogen replacement therapy in postmenopausal women has proved effective including cardiovascular disease (Massaeli and Pierce, 1995; Schwartz et al., 1995), cancer (Dreher and Junod, 1996; Vogel, 1996), and AD (see Introduction). Data are accumulating which suggest neuroprotective actions of estrogens in animal models of ischemic stroke and traumatic brain injury. For example, brain damage in rats subjected to cerebral ischemia was significantly less in females than in males, which may be related to circulating estrogen levels (Hall et al., 1991). Estrogens lessened brain damage and improved behavioral outcome in adult male rats subjected to traumatic brain injury (Emerson et al., 1993). Progesterone, which we found protects cultured hippocampal neurons against oxidative injury (Goodman et al., 1996) and synaptosomes against oxidant-induced impairment of glucose transport (present study), was reported to protect adult rats against traumatic brain damage (Roof et al., 1994). The mechanism(s) underlying the neuroprotective actions of Est and progesterone in vivo remain to be determined. Finally, antioxidant actions of estrogens in synapses may contribute to their reported effects on structural and functional synaptic plasticity. For example, Woolley and McEwen (1994) have shown that synaptic spine density in the hippocampus, which varies during the estrus cycle, is regulated by estrogens. Others have shown that long-term potentiation of synaptic transmission in the hippocampus is enhanced during proestrus (Warren et al., 1995). Our data therefore suggest the possibility that such synaptic changes might be regulated by local actions of steroids at synapses.

In contrast to estrogens and progesterone, glucocorticoids exacerbate neuronal damage induced by a variety of insults (Elliott et al., 1993; Goodman et al., 1996; Smith-Swintosky et al., 1996). In the present study, corticosterone did not modify $FeSO_4$-induced impairment of glucose transport, but did significantly exacerbate $FeSO_4$-induced impairment of glutamate transport in synaptosomes. Previous studies have shown that corticosterone exacerbates oxidative and excitotoxic injury, and inhibits glucose transport in cultured neurons (Horner et al., 1990; Goodman et al., 1996; Behl et al., 1997); the present findings are the first to show a direct endangering action of a glucocorticoid at the level of the synapse. As with the protective effects of estrogens, the potentiation of synaptic damage by corticosterone is apparently not mediated by a classic cytosolic receptor transcription factor, because the effects were observed in a neuronal compartment (synaptosomes) lacking a nucleus. Previous studies have shown that glucocorticoids can enhance depolarization-induced calcium influx in synaptosomes (Sze and Yu, 1995), although the mechanism was not examined. Additional studies will be required to establish whether synaptic actions of glucocorticoids are mediated by specific membrane receptors (Joels, 1997) or by non-specific effects on membrane properties (Mattson and Mrotek, 1985). Nevertheless, the ability of glucocorticoids to enhance synaptic oxidative stress suggests important roles for synaptic actions of glucocorticoids in neuronal endangerment (Sapolsky, 1990; Landfield et al., 1992).

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