Increasing evidence indicates that there are reductions in estrogen and androgen levels in aged men and women. These hormonal reductions might be risk factors for cognitive impairments and the development of Alzheimer’s disease (AD). Aged people show improved cognition after treatments with sex steroids. Therefore, ongoing clinical AD trials have been designed to evaluate the potential benefits of estrogen therapy in women and testosterone therapy in men. Apolipoprotein E (apoE) plays an important role in the metabolism and redistribution of lipoproteins and cholesterol. The three major human apoE isoforms, apoE2, apoE3, and apoE4, differ in their effects on AD risk and pathology. Here I review various mechanisms proposed to mediate the differential effects of apoE isoforms on brain function and highlight the potential contribution of detrimental isoform-dependent effects of apoE on androgen- and androgen receptor (AR)-mediated pathways. I also discuss potential interactions of androgens with other AD-related factors.

Introduction
Apolipoprotein E (apoE) plays an important role in the metabolism and redistribution of lipoproteins and cholesterol. The three major human apoE isoforms, apoE2, apoE3, and apoE4, are encoded by distinct alleles. They differ in having cysteine (Cys) or arginine (Arg) at positions 112 and 158 and vary in their metabolic properties (1). ApoE2 (Cys112 and Cys158) binds deftectively to low-density lipoprotein (LDL) receptors. ApoE3 (Cys112 and Arg158) binds normally to LDL receptors and is associated with normal lipid metabolism. ApoE4 (Arg112, Arg158) binds normally to LDL receptors but is associated with elevated cholesterol levels. Whereas apoE3 shows a preference for high-density lipoprotein (HDL), apoE4 shows a preference for very low-density lipoprotein (VLDL).

In the brain, apoE has been implicated in development, regeneration, neurite outgrowth, and neuroprotection (1). Compared with ε2 and ε3, ε4 increases the risk of developing Alzheimer’s disease (http://sageke.sciencemag.org/cgi/content/full/2003/43/oa2) (AD) (2-4). ApoE is associated with the pathological hallmarks of AD (http://sageke.sciencemag.org/cgi/content/full/2001/1/dn2), and the severity of AD pathology is influenced by the apoE genotype. The ε4 genotype also has a detrimental impact on AD pathology when it occurs in patients with other conditions, including progressive supranuclear palsy (PSP) (5) and Down syndrome (DS) (6). Although the ε4 allele frequency is similar in control study participants with no neurodegenerative condition and in PSP patients with minimal or no AD pathology, it is significantly higher in PSP patients who have concomitant AD pathology or the pathological signs of normal aging. The detrimental effects of ε4 are not limited to AD. Compared with ε2 and ε3, ε4 also increases the risk of developing cognitive impairments after neurotrauma (7), ischemia (8, 9), cardiopulmonary bypass surgery (10), and human immunodeficiency virus (HIV) infection (11), as well as cognitive impairments that occur with normal aging (12) and in the context of Parkinson’s disease (http://sageke.sciencemag.org/cgi/content/full/2001/1/re1) (13, 14). In multiple sclerosis patients, ε4 is associated with a worsened disease progression (15-18). Acquired immunodeficiency syndrome (AIDS) patients who develop cancer have a higher frequency of ε4 than those who do not (19). In contrast, apoE4 might be protective against liver damage caused by the hepatitis C virus (20). These results indicate that apoE affects fundamental biological processes not unique to AD.

The physiological and pathological importance of apoE have been defined using a variety of genetically altered mice, including mice that are deficient in mouse apoE (Apoε−/−), Apoε−/− mice that express one of the human apoE isoforms in the brain under the control of neuron- or astrocyte-specific promoters, and Apoε−/− mice that express one of the human apoE isoforms under the control of the endogenous mouse apoE promoter (Fig. 2). Apoε−/− mice show no obvious alterations in brain development but do show age-dependent structural and functional brain alterations. A major insight gained from comparing Apoε−/− mice without human apoE with Apoε−/− mice expressing apoE4 was that for various, but not all, measures, having apoE4 is worse than having no apoE at all, which is consistent with a pathogenic gain of function of apoE4.

Various mechanisms have been proposed to mediate the differential effects of apoE isoforms on brain function (Fig. 3). The isoform-dependent effects of apoE can be divided into the following categories: (i) effects on cholesterol transport; (ii) effects on the metabolism of amyloid β peptide (Aβ) (a key constituent of extracellular plaques found in the brains of AD patients); (iii) effects on cell signaling by lipoprotein receptors; and (iv) effects of apoE and/or proteolytic fragments on the function of cellular, including cytoplasmic, proteins to which they bind. Here I review some of these mechanisms and discuss apoE4’s potential contribution of detrimental effects on androgen- and androgen receptor (AR)-mediated pathways.

Isoform-Dependent Biological Effects of apoE
Neurite outgrowth and expression of synaptic markers
After brain injury, apoE in the nervous system has been implicated in efforts to restore neuronal function by remodeling of neuronal connections (21-23). The remodeling process may involve the effects of apoE on cholesterol and phospholipid metabolism (1, 24); on lipid efflux from both astrocytes and neurons, which results in the generation of HDL-like particles (25); on intracellu-
lar trafficking of molecules (26, 27); or on the acceleration of microtubule assembly (28, 29). For example, glial cells, a major source of apoE (24, 30), might recycle cholesterol from neuronal membranes by packaging it with apoE in order to facilitate neuronal uptake; the recycled cholesterol can then be used to promote the growth of new neuronal processes (neurite outgrowth) (21, 23, 24). ApoE-cholesterol complexes can also stimulate synapse formation in neuronal cultures (31).

The ability to remodel neurons is apoE isoform-dependent, as it is impaired in AD patients with an ε4 allele. These patients show reduced dendritic remodeling of pyramidal and subcortical neurons, whereas AD patients with two ε4 alleles show a shift toward proximal (situated toward the point of origin) branching and do not demonstrate dendritic growth in response to neuronal loss (32). Using recombinant apoE in the presence or absence of other lipoproteins (such as VLDL), apoE from transfected cells, or apoE from transgenic mice carrying the human APOE gene, the differential ability of apoE isoforms to remodel neurons by supporting neurite outgrowth (either modulating the length of the longest neurite or the total length of all neurites of a neuron) has been reported in Neuro-2A cells, a mouse neuroblastoma cell line; dorsal root ganglion cells; and primary cortical neurons; and in mouse organotypic hippocampal slice cultures (33-36). These effects of apoE on neurite outgrowth are mediated by the binding of apoE to the LDL receptor-related protein (LRP) (33, 37). Although apoE3 increased neurite outgrowth, in most (but not all) studies, apoE4 either had no effect or decreased neurite outgrowth, as compared to minus-apoE controls, and the apoE4 effect dominated over that of apoE3 when both were present in the culture (34, 37). The lack of an effect of apoE4, as compared to minus-apoE controls, and the dominant effect of apoE4 over apoE3 was also seen in vivo when age-dependent reductions in the amount of immunoreactive synaptophysin—a marker of synapses—in presynaptic terminals were quantified in the brains of transgenic mice expressing human apoE isoforms (38). These effects were also observed when neuritic sprouting was quantified in organotypic hippocampal slice cultures made from the brains of transgenic mice expressing human apoE (35).

Differential effects of apoE isoforms on synaptophysin-im-

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**Fig. 1.** ApoE and lipid metabolism. Dietary fat is packed together with apolipoproteins into chylomicrons. After entry in the circulation, the apolipoprotein composition of these particles changes, and they become enriched in apoE. During lipolysis on the surface of endothelial cells by lipoprotein lipase (LPL), which hydrolyzes triglycerides, the chylomicrons become smaller (chylomicron remnants) and fatty acids are released for energy consumption. Chylomicron remnants can be lipolyzed by LPL or hepatic lipase (HL), and lipoproteins can be taken up by the LDL receptor, the LRP receptor, the heparin sulfate proteoglycan (HSPG)/LRP complex (either binding and uptake by this complex or binding to HSPG followed by transfer to LRP for uptake), or HSPG alone. Cholesterol can be eliminated through the bile. VLDL secreted from the liver can be lipolyzed to intermediate-density lipoprotein (IDL). IDL can be hydrolyzed and converted into LDL, whereby it loses apoE. LDL particles are cleared by the liver and by cells from the reticuloendothelial system, such as macrophages, in the subendothelial space. HDL, which contains apoE, is involved in the transport of cholesterol from peripheral tissues to the liver. Esterified cholesterol can also be transferred from HDL by the cholesteryl ester transfer protein before transport to the liver.
munoreactive presynaptic terminals were also observed in bi-
genic mice that express human apoE and human amyloid precur-
sor protein hAPP (http://sageke.sciencemag.org/cgi/genedata/
sagekeGdbGene;197) in a murine apoE-deficient background
(39). ApoE3 was able to delay the age-dependent decline in
synaptophysin-immunoreactive presynaptic terminals in the
hAPP/apoE bigenic mice; at 6 to 7 months of age, hAPP/apoE3
mice had more synaptophysin-immunoreactive presynaptic ter-
minals in the neocortex than did hAPP/apoE4 mice, and, at 12
to 15 months of age, the difference also was evident in the hip-
pocampus and neocortex. As expected from the human data, 
apoE2 was neuroprotective and prevented the reduction of den-
dritic spine density in the hippocampi of young PDAPP mice, 
which express the hAPP transgene that contains the Swedish
double mutation driven by the platelet-derived growth factor-β
(PDGF-β) promoter, and of Tg2576 mice, which express the
same hAPP transgene driven by the prion protein promoter (40).

Differences in endosomal trafficking might contribute to the 
isoform-dependent effects of apoE on neurite outgrowth and the 
expression of synaptic markers. Compared to apoE3, a higher
percentage of the total apoE4 was found to be colocalized with

cathepsin D, an acidic hydrolase enriched in late endosomes and
lysosomes (41, 42) in neurons (87% in apoE4 neurons versus
9% in apoE3 neurons) and astrocytes (41% in apoE4 astrocytes
versus 33% in apoE3 astrocytes) (43) The low amount of colo-
calization of apoE3 with cathepsin D is consistent with the re-
ported higher retention of apoE2 and apoE3 than apoE4 in Neu-
ro-2A cells (26). It does not appear that more apoE4 than apoE3
is degraded; some studies report more release of apoE4 than
apoE3 in the medium, suggesting an increase in secretion. Di-

dferential recycling of the endocytosed apoE isoforms to the
plasma membrane or the interaction of apoE-containing vesi-
cles with microtubules (44) could contribute to the reduced
neurotrophic effects of apoE4. Although apoE isoforms traffic
similarly through early endosomes, apoE4, but not apoE3, con-
tinues on to late endosomes and lysosomes. After global cere-
bral ischemia in humans, isoform-dependent effects of apoE on
endocytic pathway activity were observed, with reduced endo-
cytic recycling in the presence of apoE4 (45). In Neuro-2A

cells, more apoE4 than apoE3 is secreted into the medium (26),
which might contribute to the higher concentrations of apoE4
than apoE3 (60 versus 40%) in the cerebrospinal fluid (CSF)
of human patients who carry one ε3 and one ε4 allele (46).

The differential effects of apoE isoforms on endosomal traf-
ficking are pertinent to AD. Postmortem AD brains reveal high
endocytic activity (47) with increased endosomal volumes in ε4
carriers over ε3 carriers (48). In addition to apoE, endosomal
trafficking is important for other AD-related factors, including
APP, Aβ, cholesterol, nerve growth factor (NGF), and LRP (49,
50). Endosomal abnormalities are the earliest AD pathology, ac-
companying the early rise in soluble Aβ but preceding Aβ depo-
sition, and are detected before birth in people with DS (50, 51).

Plaques and tangles

Senile plaques and neurofibrillary tangles are well-character-
ized hallmarks of AD. Senile plaques are made of Aβ, dystrophic
neurites, and reactive glial cells, whereas neurofibrillary tangles
are bundles of abnormal paired-helical filaments containing
mainly hyperphosphorylated tau (τ) protein. In the brains of AD
patients, apoE is associated with Aβ peptides in extracellular
plaques, intracellular neurofibrillary tangles, and cerebral vessel
congophilic angiopathies, a condition characterized by the depo-
sition of abnormal amyloid protein in blood vessel walls, which
can cause cerebral hemorrhage in the elderly (52). The severity
of AD pathology is influenced by the apoE genotype. For ex-
ample, the presence of an ε4 allele increases the rate and extent
of amyloid deposition over that seen in the presence of other apoE
alleles (12, 53, 54). The various apoE isoforms bind Aβ with
high avidity, but differ in their Aβ binding characteristics de-
pending on what lipids are present in their vicinity (12). There-
fore, these distinct, lipid-dependent, Aβ binding characteristics
may contribute to the isoform-dependent effects of apoE on ex-
tracellular amyloid deposition and clearance. Lipid-free apoE4
forms more effective, denser, sodium dodecyl sulfate (SDS)-
and guanidine hydrochloride-stable complexes with Aβ peptides,
and does so more rapidly, than apoE3. In addition, lipid-free apoE4
enhances zinc- and copper-induced Aβ aggregation to a greater
extent than does lipid-free apoE3 (12, 22, 55-57). These prop-
ties might explain why the ε4 allele enhances Aβ deposition
more than the ε3 allele. Also, lipid-bound apoE4 shows weaker
Aβ peptide binding than does lipid-bound apoE3 and thus might
reduce Aβ clearance (58, 59).

The C-terminal domain of apoE binds Aβ with high affinity
(12, 60, 61), can be recovered from plaques from the brains of
AD patients (61), and may promote plaque formation; C-termi-
nal apoE fragments form amyloid-like fibrils in vitro that are
positive for staining with Congo red, an agent used to detect
amyloid. There are no sequence differences among the C-termi-
nal regions of the apoE isoforms, but there might be differences
in the in vivo amount of C-terminal fragments that the various
isoforms generate; in vitro, apoE4 is more prone to proteolytic
clavage than is apoE3 (62).

Consistent with the human data, apoE has isoform-depend-
ent effects on plaque formation in murine apoE-deficient
transgenic mice that express hAPP with familial AD mutations
and human apoE isoforms in astrocytes (63) or neurons (39).
Supporting the hypothesis that apoE has isoform-dependent ef-
facts on Aβ deposition and/or clearance, mice carrying the
apoE3 isoform showed reduced Aβ deposition as compared to
mice that expressed the apoE4 version. These isoform-depend-
ent effects of apoE on plaque loads did not correlate with iso-
form-dependent differences in the number of synaptophysin-im-
munoreactive presynaptic terminals (39) or cognitive function
(64), suggesting that apoE also has plaque-independent effects
on brain function in AD.

ApoE binds to the tau protein, and the differential ability of
apoE isoforms to bind tau (12) may contribute to the isoform-de-
dependent effects of apoE on neurofibrillary tangle pathology.
For example, in young patients (aged 22 to 46 years; mean age
38 years) with initial neurofibrillary pathology (stage I), the percent-
age of ε4 alleles is higher than in control participants (65). The ε4
percentage also correlates with the extent of neurofibrillary
pathology in elderly people without dementia (66) and with the
extent of neurofibrillary pathology in AD patients (67). Isoform-
dependent effects of apoE on the microtubule-associated proteins
tau and MAP2c could contribute to their effects on neurofibril-
lar pathology (12). Expression of only the human apoE4 iso-
form in neurons, but not in non-neuronal cells, of transgenic
mice, correlated in a dose- and age-dependent manner with in-
creases in tau hyperphosphorylation. The apoE4-expressing ani-

mals showed a large number of inclusions in the neocortex, hip-
pocampus, and amygdala that contained apoE4, hyperphosphory-
lated tau, and ubiquitin (http://sageke.sciencemag.org/cgi/con-
Antibodies that recognize N-terminal apoE epitopes react with tangles (56, 69, 70), and the N-terminal fragments of apoE are more neurotoxic than those of apoE3 (71-75). A lesser extent than N-terminal apoE fragments, C-terminal apoE fragments accumulate in neurofibrillary tangles in AD brains (62), and these fragments may also contribute to tangle formation; transgenic mice that express C-terminal fragments of human apoE4 show tangle-like pathology (62).

However, the evidence for isoform-dependent effects of apoE on neurofibrillary pathology is weaker than for amyloid deposition (12, 53, 65, 66). Interaction of apoE with Aβ and tau could occur by distinct mechanisms, which would explain why AD patients with the ε4/ε2 genotype show significantly less amyloid deposition, but not less neurofibrillary pathology, in the neocortex than those with the ε4/ε3 genotype. AD patients with the ε4/ε2 and ε3/ε2 genotypes showed opposite effects on amyloid load, neuritic plaques, and neurofibrillary tangles (76).

The differential effects of apoE isoforms on AD-like pathology is also observed in PSP and DS patients. The ε4 allele frequency is significantly higher in PSP patients with concomitant AD pathology (64%) or with pathological aging (many cortical senile plaques, mostly diffuse amyloid deposits, and few or no neurofibrillary tangles) (38%) (17). In addition, ε4-positive PSP patients have significantly more senile plaques and neurofibrillary tangles in association cortices (cortical areas that are neither motor nor sensory but are thought to be involved in higher processing of information) and more senile plaques in primary cortices than do ε4-negative PSP patients.

ApoE and nitric oxide production

ApoE and nitric oxide production

ApoE-Aβ interactions

ApoE, hyperlipidemia, and atherosclerosis

ApoE and AD

Fig. 2. Mouse models used for studying the physiological and pathological roles of apoE.

dogenous androgens as compared to males, female mice that express human apoE4 might be more susceptible to the effects of reduced AR binding on cognitive function. However, when male mice that express either human apoE3 or apoE4 were treated with the AR antagonist hydroxyflutamide (87), striking spatial learning and memory impairments were seen only in the male mice that expressed apoE4 (86). There was no difference in plasma concentrations of testosterone in male apoE3- and apoE4-expressing mice, indicating that plasma testosterone does not contribute to the differential effects of hydroxyflutamide on cognitive performance in male apoE3- and apoE4-expressing mice. In contrast to the situation with mice, in humans, low serum testosterone concentrations and the interaction of testosterone with apoE4 might each increase one's risk of developing AD (88, 89). This effect might be gender-dependent, because in postmenopausal women, those with an ε3/ε4 genotype
have higher serum testosterone concentrations than those with an ε3/ε3 genotype (90).

Role of ARs in spatial learning and memory

These data predict that ARs play a role in spatial learning and memory. To assess this prediction, we studied mutant mice with a naturally occurring defect in the AR gene (testicular feminization mutant or tfm) (91, 92). Because the trait in tfm mice is X-linked, males are completely androgen-insensitive and females are partially insensitive. If ARs are important for spatial learning and memory, female tfm carrier mice would be expected to outperform tfm male mice in tasks that require a spatial map of the environment to locate a hidden platform in a pool of opaque water (see Video 1 http://sageke.sciencemag.org/cgi/content/full/2004/11/re2/DC1). With training, the tfm female and male mice improved their performance in the visible and hidden platform tasks (there was a significant effect of session, P < 0.01). Although there was no difference in the ability of the tfm female and male mice to locate the visible platform, tfm female mice were better than tfm male mice at locating the hidden platform (P < 0.01, Tukey-Kramer posthoc test). Both groups of mice swam continuously and in similar patterns, and there were no significant periods of passive floating. In the probe trial (platform removed), both groups showed spatial memory retention and spent more time in the quadrant of the pool that used to contain the hidden target location (target quadrant) than in any of the other quadrants (P < 0.01, Tukey-Kramer posthoc test); however, tfm female mice spent more time in the target quadrant than did tfm male mice (P < 0.05, Tukey-Kramer posthoc test). We excluded the possibility that the variations in water maze performance resulted from differences in sensorimotor function (see Video 2 http://sageke.sciencemag.org/cgi/content/full/2004/11/re2/DC1) or anxiety levels (see Video 3 http://sageke.sciencemag.org/cgi/content/full/2004/11/re2/DC1). The fact that female tfm carrier mice outperformed tfm male mice in the water maze supports the hypothesis that ARs have an important role in spatial learning and memory.

Males are only partially protected against the effects of apoE4

The finding that expression of apoE4 in Apoe<sup>−/−</sup> mice leads to an age-related impairment of spatial learning and memory only in females is consistent with the observed epidemiological relation between apoE4-expressing human females and an increased risk of developing AD. However, the crossing of apoE4-expressing transgenic mice with transgenic mice that express a version of the hAPP gene that contains well-characterized AD mutations revealed that the male mice are only partially protected against the detrimental effects of apoE4. Although 6-month-old male hAPP/apoE3 mice showed spatial memory retention, male hAPP and hAPP/apoE4 mice did not (64). Consistent with the stimulation of AR in wild-type brains (93, 94), androgens and AR-dependent pathways protected female mice against the detrimental effects of apoE4 on spatial learning and memory, and this protection was associated with increased cytosolic AR binding (86).

ApoE4, AR, and cholinergic function

The cholinergic basal forebrain participates in behavioral processes such as attention and memory, functions that decline with age. The negative effects of apoE4 on AR function might also extend to alterations in the cholinergic basal forebrain that are associated with aging and AD (95). In the vertical limb of the diagonal band of Broca, a major cholinergic nucleus in the basal forebrain that is affected in AD, the presence of either AD pathology or an APOE ε4 allele negatively correlates with the percentage of AR-positive neurons in women but not in men.

![Fig. 3. Possible mechanisms that mediate the isoform-dependent effects of apoE.](http://sageke.sciencemag.org/cgi/content/full/2004/11/re2)

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ApoE4, AR, and oxidative stress

The detrimental effects of apoE4 on AR function might not be limited to the brain. Peritoneal macrophages were isolated from transgenic mice that expressed either human apoE3 or apoE4 under the control of the mouse Apoe promoter and stimulated with interferon-γ either alone or in combination with synthetic double-stranded RNA (Poly I:C) or lipopolysaccharide (LPS). Peritoneal macrophages from male apoE4 homozygous mice showed a greater release of the inflammatory mediators nitric oxide and tumor necrosis factor-α (TNF-α) than did those from male apoE3 homozygous mice (96, 97). This isoform-dependent effect of apoE on the release of nitric oxide was gender-dependent and was not seen in peritoneal macrophages from female apoE3 and apoE4 homozygous mice.

On the basis of these results, as well as the reported increase in nitric oxide synthase (NOS) activity in the brains of rats after castration and the decrease of NOS activity after administration
of the dihydrotestosterone (98), Sing et al. decided to evaluate the potential role of androgens in the isoform-dependent effects of apoE in peritoneal macrophages derived from apoE transgenic male mice. They found that, after stimulation with IFN-γ + LPS, TNF-α release was higher in peritoneal macrophages from castrated apoE3 homozygous mice than in macrophages from sham-treated or castrated + testosterone-treated apoE3 homozygous mice; in contrast, there was no difference in IFN-γ + LPS-stimulated TNF-α release in peritoneal macrophages from castrated and castrated + testosterone-treated apoE4 homozygous mice (97). These findings show that apoE4 reduces the sensitivity of AR-mediated signaling.

The increased release of nitric oxide in stimulated peritoneal macrophages and microglia-immune cells from the central nervous system isolated from apoE4 transgenic mice (96) might contribute to the increased vulnerability of apoE4-expressing organisms to Aβ-induced oxidative stress (http://sageke.sciencemag.org/cgi/content/full/2001/1/oa5). (protein oxidation, lipid peroxidation, and the generation of reactive oxygen species) (99). The differential abilities of apoE isoforms to bind, via their cysteine residues, to the toxic lipid peroxidation product 4-hydroxynonenal (HNE) might also contribute to the increased vulnerability of apoE4-expressing organisms to Aβ-induced oxidative stress (100). Treatment of rat hippocampal cultures with Aβ peptides leads to increases in the amounts of free and protein-bound HNE (101), and the differential abilities of the apoE isoforms to bind HNE correlate with their abilities to protect against apoptosis (apoE2 > apoE3 > apoE4) (100).

ApoE4 and estrogen

Some of the biological effects of testosterone require aromatization of testosterone to estrogen, and such effects might be partially or completely mediated by estrogen receptors (ERs). This observation raises the following question: Could increased ER function compensate for reduced AR function in the presence of apoE4? This possibility is unlikely. Clinical data indicate that estrogen has a beneficial effect on aspects of cognitive function only in healthy elderly women who do not carry the e4 allele; no such effect has been observed in healthy elderly women that carry e4 (102) or in women with AD. Together these data suggest that, with regard to cognitive function in the presence of apoE4, ARs might be more responsive to androgens (86) than ERs to estrogens (102). Because 17β-estradiol, but not dihydrotestosterone, up-regulates apoE gene expression (103), the interaction between apoE and ERs might be more complex than the one between apoE and ARs.

AR CAG Repeat Polymorphism and AD Risk

The human AR gene contains eight exons that encode a 110-kD member of the nuclear receptor superfamily, a collection of proteins that bind to DNA in a sequence-specific manner and regulate gene transcription (Fig. 4) (104). The first exon encodes the major transcriptional activation function (AF-1) and the N-terminal domain, which contains polymorphisms that result from variable numbers of glutamine (11-35) and glycine (10-31) repeats. Although shortening of the polylutamine or polyglycine stretch is associated with a predisposition to prostatic neoplasia (105), expansion of the glutamine repeat can cause sequestration of transcriptionsal coactivators such as the CREB-binding protein (CBP), resulting in age-dependent spinal and bulbar muscular atrophy (106, 107). AF-1 interacts with the glutamine-rich region of steroid receptor coactivator-1 (SRC-1) (108), which in turn can interact with the global activator CBP/p300; together, these interactions stimulate transcriptional activation by the AR in a synergistic manner (109). CBP/p300 can also interact directly with ARs.

Altered AR function that results from a polymorphism in the glutamine (CAG) repeats in exon 1 of the AR gene might also increase AD risk. Within the normal range of CAG repeats, alleles with 20 or fewer repeats have been shown to be associated with an increased risk for AD in men but not in women (110). Although the association appeared to be stronger in men lacking the e4 allele, logistic regression did not reveal an interaction between e4 and the short AR alleles. Because polymorphisms in the CAG repeats correlate with altered transcription (111) and translation (112) of the AR gene and gene product, the diminished AR function observed in men with such polymorphisms might be similar to that seen in men who carry the e4 allele.

The second and third exons of the AR gene encode the DNA binding domain (DBD), which contains two zinc fingers that enable ARs to bind, in a sequence-specific manner, to androgen response elements (AREs), which are cis-acting regulatory DNA elements in target genes. Whereas the N-terminal zinc finger is involved in sequence-specific binding, the C-terminal zinc finger is involved in stabilization of the AR-DNA complex (113). Exons 4 to 8 encode the ligand-binding domain (LBD) and a minor transcriptional activation function (AF-2). AF-1 and AF-2 both interact with the leucine-rich region of SRC-1 (108). Phosphorylation by mitogen-activated protein kinase (MAPK), which is stimulated by androgens, regulates the activity of SRC-
tion of ARs and requires the presence of IL-6 (114).

**Nuclear translocation**

ARs not bound to hormone are localized in the cytoplasm as a complex with heat shock proteins and immunophilins—intracellular proteins that bind immunosuppressive drugs. When the endogenous androgens testosterone or 5α-dihydrotestosterone (DHT) bind to the AR, it changes its confirmation, dissociates from the complex by releasing the associated proteins, forms homodimers, and unmasks its nuclear localization signal. Once unmasked, this signal binds importins, which transport the hormone-bound AR into the nucleus. In the nucleus, active hormone-bound ARs enter transcriptionally active nuclear foci, whereas antagonist-bound ARs enter the nucleus but do not enter the active nuclear foci. Selective AR modulators (SARMs) alter AR function either by failing to translocate ARs to the nucleus or by translocating ARs to the nucleus but not into the active nuclear foci.

ARs can modulate gene transcription by binding to AREs but are also able to activate and repress transcription of the AR gene and genes encoding other transcription factors without interacting directly with DNA. This is accomplished by the rapid stimulation of second-messenger cascades by ARs, which results in the direct phosphorylation (and activation) of transcriptional activator proteins. The calcium-binding protein calreticulin is able to dissociate AR from the DNA by competing for the DBD of the AR and may export AR back to the cytoplasm for a subsequent round of nuclear translocation.

**Interaction of Testosterone with AD-Related Factors**

Increasing evidence supports the notion that androgens have neuroprotective effects. Although testosterone is not an antioxidant by itself, as it lacks the phenol group present in 17β-estradiol, it decreases the susceptibility of cells to oxidative stress (115, 116). In addition, DHT reduces neuronal vulnerability in the CA2/3 areas of the hippocampus of gonadectomized rodents to a challenge with the excitotoxin kainite (117). These effects are likely mediated by ARs and not by the DHT metabolite 3α-androstenediol, which does not bind AR and is suggested to have anti-seizure effects; DHT does not reduce seizure severity, indicating that the neuroprotective effects of DHT are independent of the modulation of seizure activity (117). Also, in contrast to DHT, 3α-androstenediol does not provide neuroprotection against Aβ toxicity in cultured hippocampal neurons (118). Consistent with the hypothesis that the neuroprotective effects of androgens are mediated by ARs, DHT increased dendritic spine density in the CA1 area of the hippocampus in ovariectomized female rodents (119), and testosterone and the nonaromatizable androgen mibolerone, when given in the presence of aromatase inhibitors, protected against serum depriva-
tion-induced apoptosis in human primary fetal neurons (120).

Testosterone, APP, and Ab peptides

The rest of this review focuses on the interaction of testosterone with AD-related factors (Fig. 5) and the relevance of evaluating potential isoform-specific effects of apoE on androgen and AR-related pathways. Cleavage of APP by α-secretase results in the formation of nonamyloidogenic soluble α APP (sAPPα), which has neurotropic properties. Cleavage of APP by β-secretase results in the formation of soluble β APP (sAPPβ); cleavage of sAPPβ by γ-secretase results in the formation of the amyloidogenic species Aβ. A single dose of testosterone increases the secretion by hypothalamic cells of a collection of soluble versions of the APP protein, mostly sAPPα, through a mechanism involving the MAPK pathway (121). Because treatment of these cells with the AR blockers flutamide and cyproterone acetate does not completely inhibit the effects of testosterone on sAPP secretion [it is known that flutamide does not disrupt and even mimics some nongenomic effects of ARs (122, 123)], it is unclear whether ARs are required for the observed effect. Because aromatase inhibition suppresses the effect of testosterone on sAPP secretion, and estrogens increase sAPPα secretion through a mechanism involving MAPK, conversion of testosterone to estrogen contributes to this effect. In addition to increasing the release of sAPPα, testosterone may also exert its neuroprotective effects by reducing the secretion of Aβ peptides and Aβ-induced neuronal toxicity (124, 125).

Testosterone and tau phosphorylation

The τ gene encodes multiple phosphorylated and thermostable versions of the τ protein by alternative splicing. The triggers of the dephosphorylation-hyperphosphorylation cycle that leads to the formation of the paired helical τ filaments are unknown, but it has been suggested that these events represent a defense against stressors. Heat shock induces hyperphosphorylation of τ by glycogen synthase kinase-3β (GSK-3β), a proapoptotic kinase that inhibits the protective effects of the heat shock response by inhibiting signal transduction cascades that protect neurons against programmed cell death. Testosterone, but not 17β-estradiol, inhibits heat shock-induced hyperphosphorylation of τ by preventing the hyperstimulation of GSK-3β (126). Thus, testosterone might be neuroprotective by inhibiting the phosphorylation of τ.

AR and β-catenin

Increasing evidence indicates a link between ARs and β-catenin (127-129). β-catenin is localized in the plasma membrane of cells, where it regulates cell-cell contacts, and in the nucleus and plasma membrane, where it controls the Wnt signaling pathway (http://stke.sciencemag.org/cgi/cm/stkecm; CMP_12420), which is involved in polarity, cell proliferation, and development. In the nucleus, β-catenin forms a complex with the T cell factor/lymphoid enhancer factor family of transcription factors to activate transcription. In the absence of signaling, cytoplasmic β-catenin is phosphorylated by GSK-3β and subsequently undergoes proteosomal degradation.

β-catenin lacks a nuclear localization signal and thus requires the participation of accessory proteins that chaperone β-catenin into the nucleus. Agonist- but not antagonist-bound AR interacts with β-catenin (127), suggesting that ARs may escort β-catenin to the nucleus. Compared to full-length AR, truncated AR mutants that lack the C-terminal LBD show enhanced nuclear localization of β-catenin in the absence of 5α-DHT, but incomplete nuclear localization in the presence of 5α-DHT. These data indicate that the C terminus of AR is required for proper regulation of β-catenin nuclear localization. In neuronal cells, hormone-bound ARs that colocalize with β-catenin in the nucleus repress its ability to activate transcription; conversely, β-catenin represses AR stimulation of ARE-mediated transcriptional activation.

Nuclear receptors other than ARs also interact with β-catenin. ERs modulate the activity of β-catenin indirectly by interacting with the T cell factor/lymphoid enhancer factor family of transcription factors. Depending on the collection of factors present, activation of transcription by ERs is either enhanced or repressed.

In addition to nuclear factors, the transmembrane protein presenilin-1 (PS-1) (http://sageke.sciencemag.org/cgi/gedata/sagekeGdbGene;198) is also associated with β-catenin (130, 131). PS-1 is part of the γ-secretase protein complex that cleaves APP. The balance between ARs and β-catenin may be important under both physiological and pathological conditions. PS-1 was shown to reduce the stability and transcriptional activation activity of β-catenin, whereas PS-1 that contained familial AD mutations, which are responsible for most cases of early-onset autosomal dominant familial AD, was less able to do so. Consistent with these data, PS-1 deficiency has been associated with increased stability of cytosolic β-catenin. Because β-catenin antagonizes the actions of ARs in the nucleus, PS-1 containing familial AD mutations might reduce AR function by increasing the stability of β-catenin and thus contribute to AD.

NGF neurotransmission

Impaired NGF signaling has been proposed as a mechanism that contributes to AD. The cholinergic basal forebrain neurons are dependent on NGF and its receptors for their survival. Testosterone might improve NGF signaling, because it increases its release and up-regulates the p75 NGF receptor in brain areas relevant for cognition, including the hippocampus. Exposure of young apoE transgenic mice to a more complex (enriched) environment increases NGF concentrations in the hippocampus of apoE3- but not apoE4-expressing mice (132), suggesting that apoE isoforms might have differential effects on testosterone-mediated NGF signaling.

Testosterone and serotonin (5-HT)

Altered serotonin 5-HT2A receptor signaling might be pertinent to AB plaque formation, as activation of 5-HT2A receptors increases APPs and reduces AD formation in brain slices and cultured cells (133). Testosterone and estrogen, but not DHT, increase transcription of the 5-HT2A receptor gene and the serotonin transporter gene in the forebrain (134). The lack of an effect of DHT and testosterone on transcription of the 5-HT2A receptor and serotonin transporter genes in the caudate putamen, a brain area that has little aromatase activity, indicates that conversion of testosterone to estrogen is required for these effects.

Summary

The importance of apoE in healthy and unhealthy brain function is supported by the reported isoform-dependent effects on cholesterol transport, Aβ metabolism, endosomal activity, mitochondrial activity, neurite outgrowth and synaptic markers, intracellular calcium levels, signaling by lipoprotein receptors, and the function of cytoplasmic proteins to which they bind. Some of these effects might be mediated by proteolytic fragments of apoE.

In addition to and perhaps in concert with these potential mechanisms, reduced androgen concentrations and AR-mediated
signaling may contribute to cognitive deficits in the elderly and in AD patients. The spatial learning and memory performance of fmn mice support a role for ARs in brain function and the androgen hypothesis. Androgens might be neuroprotective by antagonizing the detrimental effects of apoE4 on AR function or other AD-related factors, such as Aβ, hyperphosphorylated τ, and PS-1.

More research is needed to evaluate the potential role of interactions between apoE isoforms, AR, and gender in the various mechanisms proposed to contribute to the isoform-dependent effects of apoE on AD risk. Understanding these interactions is important for developing and evaluating the therapeutic effects of androgens and SARMs, which are tissue-selective and maintain the therapeutic effects of androgens but reduce their undesirable side effects.

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