Estrogenic Actions in the Brain: Estrogen, Phytoestrogens, and Rapid Intracellular Signaling Mechanisms

SCOTT M. BELCHER and ATTILA ZSARNOVSZKY
Department of Pharmacology and Toxicology, University of Arkansas for Medical Sciences, Little Rock, Arkansas
Received April 6, 2001; accepted May 10, 2001 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT
The endogenous gonadal steroid 17β-estradiol (E2) plays an important role in the development, maturation, and function of a wide variety of reproductive and nonreproductive tissues, including those of the nervous system. The actions of E2 at target tissues can be divided into 1) long-term “genomic” actions that are mediated by intracellular estrogen receptor-induced changes in gene expression and 2) rapid actions that modulate a diverse array of intracellular signal transduction cascades. Environmental estrogens are compounds present in the environment that can mimic, and in some cases antagonize, the effects of endogenous estrogens. As a result of these actions, there is currently much interest within the scientific community regarding the relative benefits or threats associated with exposure to different environmental estrogens. Within the general public there is considerable acceptance of the benefits associated with increased use of “natural” estrogens as a component of a healthy diet and in postmenopausal women as an alternative to estrogen replacement therapies. First, this review will focus attention on the role of estrogens in the central nervous system by briefly discussing some of the known mechanisms through which estrogen’s effects are mediated, focusing on rapid intracellular signaling mechanisms during neurodevelopment. Second, with the hope of bringing attention to an area of study that until recently has received little consideration, we will briefly discuss phytoestrogens and suggest that these compounds have the potential to influence rapid E2-induced mechanisms in the nervous system in ways that may result in modified brain functions.

Introduction
Estrogens influence growth, differentiation, maturation, and function of many different target tissues including the cells of the central and peripheral nervous system. The endogenous gonadal steroid 17β-estradiol (E2) regulates gene expression in target tissues and dramatically influences diverse physiological processes through its cognate receptors estrogen receptor (ERα) and ERβ. These receptors are members of the steroid/thyroid superfamily of transcription-factor receptors, and they share common modular domains whose structures and functions are conserved. Overall, the ERα and ERβ proteins are about 47% identical and are expressed from different genes that are located on separate chromosomes (Enmark et al., 1997). Each receptor binds E2 with high affinity (Kd = 0.1 and 0.4 nM, respectively), can interact as homo- or heterodimers with estrogen-responsive elements (ERE) or associate with the AP1 transcription factors c-jun or c-fos to influence transcription of responsive genes (Kuiper et al., 1996, 1997; Paech et al., 1997).

Numerous studies have also demonstrated that E2, as well as other steroid hormones, can rapidly (within seconds to a few minutes) influence cellular physiology in many different cell types of reproductive and nonreproductive tissues through the activation of a diverse array of intracellular signaling mechanisms. For example, E2 has been shown to rapidly activate adenylate cyclase, increase intracellular [Ca2+], activate phospholipase C to generate inositol 1,4,5-trisphosphate and diacylglycerol, stimulate nitric-oxide synthase to generate nitric oxide, and activate the extracellular regulated kinases 1/2 (ERK1/2) mitogen-activated protein kinase (MAPK) pathway (Falkenstein et al., 2000). While the molecular mechanisms of nongenomic E2 action are probably diverse and therefore not well understood, it is known that some rapid E2 effects are initiated by the binding of E2 at

ABBREVIATIONS: E2, estradiol; ER, estrogen receptor; ERE, estrogen-responsive element; MAPK, mitogen-activated protein kinase; CNS, central nervous system; CREB, cAMP-responsive element binding protein; ERK, extracellular regulated kinase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C.
membrane-associated ERs that are closely related to the "classical" intracellular receptors (Levin, 1999; Norfleet et al., 1999; Razandi et al., 1999; Watson et al., 1999). However, there is additional evidence indicating that some rapid actions of E2 are independent of the intracellular ER (Falkenstein et al., 2000; Schmidt et al., 2000).

How the diverse mechanisms through which estrogens mediate their resulting physiological effects are integrated is not known; however, it seems reasonable that the total physiological effects of E2 are the consequence of both rapid nongenomic mechanisms and longer-duration genomic mechanisms. In this regard, little is known concerning the relative contribution of genomic and nongenomic mechanisms to the integrated physiological effects of estrogens. In this brief review, we will focus our attention on the role of estrogens in the central nervous system by briefly discussing what is known concerning rapid intracellular signaling mechanisms(s) through which estrogen's effects are mediated, discuss the potential of estrogenic compounds present in the environment to influence the actions of endogenous estrogens, and suggest that these compounds may potentially modify the activities of endogenous estrogens in the nervous system.

**Rapid Mechanisms of Estrogen Action in the Nervous System.** In the brain, E2 is well known as a fundamental regulator of the physiology and behaviors required for reproduction. Along with its role in regulating neuroendocrine functions and sexual behaviors, E2 also plays a significant role during normal development and in genderization of the mammalian central nervous system (CNS), and it has important neurotrophic and neuroprotective functions in the brain (Beyer, 1999; Toran-Allerand et al., 1999; Wise et al., 2001).

Recently, much evidence concerning rapid nongenomic actions of E2 in the CNS has accumulated suggesting that these rapid effects are of major importance for normal development and function of the brain (Kelly and Wagner, 1999; Toran-Allerand et al., 1999; Woolley, 1999). Many of the signaling pathways identified as being rapidly modulated by E2 in other tissues are also rapidly modulated by E2 in the brain (Fig. 1). Numerous studies have established that the modulation of G-protein-coupled receptors is an important mechanism through which E2 acts to rapidly alter neuronal excitability. For example, E2 has been shown to rapidly cause the opening of K+ channels and to inhibit L-type Ca2+ currents in gonadotropin-releasing hormone neurons, and to activate non-NMDA-type glutamate receptors in hippocampal neurons through G-protein-coupled and protein kinase A (PKA)-dependent mechanisms (reviewed by Kelly and Wagner, 1999).

Rapid actions of E2 have also been shown to play an important role in neuronal differentiation. In developing murine midbrain dopaminergic neurons, E2 rapidly stimulates the release of Ca2+ from intracellular stores (Beyer and Raab, 1998) and can activate a PKA signaling cascade that results in activation of the CAMP-responsive element binding protein (CREB) (Beyer and Karolczak, 2000). Because these effects were mediated by both E2 or by membrane-impermeable E2 conjugated to bovine serum albumin, were blocked with cAMP/PKA antagonists and calcium depletion but were
not inhibited with ICI 182,780, it was concluded that non-
genomic activation of the calcium and cAMP/PKA signaling
cascades by E_2 is initiated at a membrane binding site and
that this E_2-induced mechanism is involved in estrogen-mediated
differentiation of midbrain dopaminergic neurons
(Beyer and Karolczak, 2000).

Other rapid actions of E_2 have also been shown to influence
additional signaling pathways resulting in increased neuronal
survival. For example, in cultured cortical neurons E_2 can
rapidly activate the phosphatidylinositol 3-kinase (Honda et
al., 2000) and MAPK signaling pathways (Singer et al., 1999;
Singh et al., 1999). Both of those E_2-activated mechanisms
have been shown to increase cell survival and to protect
cortical neurons from excitotoxic cell death. In addition to
cortical neurons, E_2 rapidly protects hippocampal neurons
from excitotoxicity through a mechanism that requires
MAPK activation (Bi et al., 2000), and recent results from our
laboratory have demonstrated that E_2 can rapidly activate
MAPK signaling in developing cerebellar neurons (Wong and
Belcher, 2000). Together, results obtained in neurons from
many different brain regions demonstrate that the rapid
actions of E_2 are pleiotropic, are not specific to reproductive
tissues, and are not specific to regions of the brain associated
with reproductive or neuroendocrine functions.

**Rapid Activation of MAPK Signaling in the Brain.**
The interaction between estrogenic activity and growth fac-
tor signaling is well established in the brain, and much
evidence indicates that neurotrophic growth factors and es-
trogen may act in concert as well as reciprocally to regulate
differentiation of their target neurons (Toran-Allerand,
1996). Because of the apparently critical role played by E_2
in modulating growth, differentiation, and viability of both neu-
rons and glia, the mechanisms through which E_2 modulates
growth factor-dependent MAPK signaling in developing
brain cells is currently the subject of intensive research
efforts. In neurons, the MAPK pathway is activated by the
binding of neurotrophins (e.g., brain-derived neurotrophic
factor, nerve growth factor) at their cognate receptor tyrosine
kinases and initiates the sequential p21Ras-mediated phos-
phorylation and activation of downstream effector kinases
(Fig. 1). Recently, the ERK1/2 MAPK signaling pathway was
shown to be rapidly activated by E_2 in rat cortical neurons in
culture (Singer et al., 1999), and a heteromultimeric complex
containing ERα and components of the MAPK cascade was
described in primary cultured cortical explants (Singh et al.,
1999). The results of the immunoprecipitation experiments
in this latter study suggest that in these cortical cells there is
a direct interaction between components of the neurotrophin-
activated and E_2-mediated MAPK signaling pathways at the
level of B-Raf (Toran-Allerand et al., 1999) (see Fig. 1).

In spite of the apparent interaction between ERα and
B-Raf in rat cortical explants, in a subsequent study it was
suggested that rapid E_2-mediated MAPK activation was in-
de-pendent of both ERα and ERβ (Singh et al., 2000). Fur-
thermore, it was found that E_2-induced MAPK phosphoryla-
tion was not abolished in murine cortical explants derived
from an ERα knockout model. Surprisingly, it was instead
found that E_2-mediated MAPK activation was potentiated
relative to control cultures derived from wild-type mice
(Singh et al., 2000). Additional experiments using the ERα
knockout cortical explant cultures indicated that MAPK sig-
aling was also activated by 17α-E_2 (a transactivationally
inactive isomer of E_2); and in contrast to wild-type controls,
MAPK activation was insensitive to the pure antagonist of
ER-transactivation ICI 182,780 (Singh et al., 2000). The find-
ing that E_2-induced MAPK activation was inhibited by ICI
182,780 in explants from wild-type mice is surprising in light
of the previous experimental results that demonstrated that
in these cultured cortical explants from wild-type rats, E_2-
induced activation of MAPK was insensitive to ICI 182,780
(Singh et al., 1999). The significance of the differences be-
tween results obtained with cortical explants derived from
rats or those obtained from wild-type and ERα knockout mice
remains unclear.

In additional experiments, the phytoestrogen genistein
was used as an ERβ-specific ligand and 16α-iodo-17β-esta-
diol was used as a specific ERα ligand. The results of those
studies indicated that neither compound induced MAPK
phosphorylation in wild-type explants (Singh et al., 2000). These
results—together with those described above—were
interpreted to suggest that rapid E_2-mediated activation of
MAPK was independent of both ERα and ERβ. It was there-
fore proposed that a novel ICI 182,780-insensitive ER was
responsible for rapid MAPK activation by E_2. When consid-
ering the significance of these results, it is important to note
that while 16α-iodo-17β-estradiol and genistein preferen-
tially bind ERα and ERβ, respectively, their pharmacological
properties as selective ER agonists or antagonists have not
been well characterized. This is especially true in relation to
their influence of rapid ER-mediated actions. As a result,
further characterization of the pharmacological properties of
these compounds (particularly in regard to rapid actions) is
needed to clarify the significance of experiments that use
them as isofrom-selective ER agonists. Thus, whether or not
cortical neurons express a novel ER that mediates the rapid
activation of MAPK signaling by E_2 remains an interesting—
although unanswered—possibility. However, if additional
studies confirm that a novel ER mediates rapid activation of
MAPK in cortical neurons, recent results suggest that a
candidate receptor may likely be one of two types: 1) an ER
that is evolutionarily related to the known intracellular ERs
(a third ER isoform, ERγ, was recently identified in teleosts;
Hawkins et al., 2000); or 2) similar to the novel plasma
membrane γ-adrenergic receptor that is expressed in neu-
rons (Yawo, 1999) and that has been shown to mediate some
nongenomic actions of E_2 and xenoestrogens in pancreatic
β-cells (Nadal et al., 2000).

**Environmental Estrogens**

Environmental estrogens are a large and structurally di-
verse group of compounds that can mimic and in some cases
antagonize the effects of endogenous estrogens, and they are
therefore often referred to as “endocrine disruptors”. As a
result of their estrogen-like activities and the potential for
some to block the normal actions of endogenous E_2, there
is currently much debate within the scientific community, and
also considerable interest within the general public regard-
ing the relative benefits or threats associated with exposure
to environmental estrogens.

Compounds characterized as having estrogenic properties
are typically divided into two general categories, the xeno-
estrogens and the phytoestrogens. Xenoestrogens are a
diverse group of synthetic compounds that include pesticides
such as 1,1,1-trichloro-2-[α-chlorophenyl]-2-[α-chlorophenyl]ethane; the widespread industrial pollutants poly-chlorinated biphenyls; bisphenol-A, which is present in canned foods and dental sealants; the synthetic estrogen, diethylstilbestrol; and many others. As a result of their negative actions on reproductive tissues, xenoestrogens are a potential threat to wildlife and human populations and are therefore the subject of much active research. Because numerous studies and recent reviews have focused on various aspects of xenoestrogen action (Tyler et al., 1998; Nilsson, 2000; Roselli et al., 2000), these compounds are addressed here only in regard to their potential as estrogenic compounds to mediate effects in the brain similar to those of phytoestrogens. It should however be pointed out that while the long-term estrogenic effects of both phytoestrogens and xenoestrogens have been extensively studied, there is a similar lack of experimental data concerning the influence of both natural and synthetic estrogenic compounds on rapid E₂-mediated mechanisms. The ability of these compounds to influence rapid actions of E₂ in the brain and how such effects may impact the normal development and physiological properties of cells in the brain is currently unknown.

The phytoestrogens are a group of naturally occurring compounds with estrogenic activity that are present in plants or that arise from bacterial or fungal metabolism of plant precursor compounds. To varying degrees, phytoestrogens can also act as agonists or antagonists of the normal actions of E₂, and in adults they may have protective effects against certain forms of cancer, cardiovascular disease, and osteoporosis and may also prevent undesirable menopausal symptoms (Bingham et al., 1998). As a result of these potentially beneficial effects, phytoestrogens, especially soy isoflavones, have increasingly gained widespread acceptance as safe and beneficial dietary components and as a “natural” alternative to estrogen-based hormone replacement therapies. This increased use of phytoestrogens has occurred even though their mechanisms of action and their effects (either positive or negative) on the developing and mature brain are not well understood. It is also of interest to note that the ready acceptance of the safety and the benefits associated with exposures to increased concentrations of the natural estrogenic compounds by the general public and the medical community is in sharp contrast to the common (and potentially accurate) perception that the actions of xenoestrogens are a threat to the health and well-being of human and wildlife populations.

In regard to human dietary exposure, there are three major classes of phytoestrogens: the isoflavonoids, the coumestans, and the lignans (Fig. 2). The most significant and therefore most well studied are the isoflavones genistein and daidzein. These phytoestrogens are regularly consumed in soy-containing food products that include infant formula and—as mentioned above—are increasingly used in the form of over-the-counter dietary supplements as an alternative to hormone-replacement therapies to relieve menopausal symptoms. Because infants that are fed soy-based formula have especially high plasma concentrations of daidzein and genistein during critical periods of brain development (Setchell et al., 1998), understanding the normal actions of E₂ during perinatal development of the brain, the way phytoestrogens may influence these actions, and whether they exert long-term effects on neuronal function is extremely important.

Lower but potentially significant concentrations of genistein and daidzein are also present in a wide variety of fruits and nuts (Liggins et al., 2000). Along with isoflavones, the coumestan coumestrol (Fig. 2), which is present in sprouts of soybeans, clover, and alfalfa, is another significant phytoestrogen regularly consumed by humans. Lignans, while receiving less research attention, are present in a wide variety of normally consumed foods and therefore represent a potentially significant source of dietary phytoestrogens. The two major mammalian lignans, enterolactone and enterodiol (Fig. 2), are absorbed as the fermentation products of gut bacterial metabolism of the precursor plant lignans ma-tairesinol and secoisolariciresinol, respectively (Bingham et al., 1998). An additional group of natural estrogenic compounds, the mycoestrogens (e.g., zearalenone; Fig. 2), are generated by metabolic actions of molds belonging to the genus Fusarium that frequently infest pasture grasses and legumes.

Some phytoestrogens have obvious structural similarity with E₂ and are typically considered to act as E₂ mimetics; however, many compounds characterized as having estrogen-like properties have few obvious structural similarities to E₂.
Phytoestrogens and Estrogen Receptors

In cells of reproductive tissues, many studies have shown that environmental estrogens typically bind ERs with low affinity and can mimic or block the actions of endogenous E$_2$. In some cases, the estrogenic effects of these compounds are thought to be protective against certain cancers and in other instances have been causatively linked with some hormone-responsive cancers (Bingham et al., 1998; Kuiper et al., 1998; Arcaro et al., 1999; Rosselli et al., 2000).

In contrast to the ability of phytoestrogens to influence reproductive tissues, much less is known concerning the mechanistic effects of these environmental estrogens on the developing nervous system, with especially little known about rapid nongenomic mechanisms of action in brain regions outside of the neuroendocrine axis. However, recent studies in smooth muscle and in a pituitary tumor cell line have demonstrated that xenoestrogens can activate rapid nongenomic mechanisms that are also activated by E$_2$ (Ruehlmann et al., 1998). Although the available evidence is currently limited, it seems probable that in other nonreproductive cell types—including those of the nervous system—xenoestrogens and phytoestrogens may have similar effects (either agonistic or antagonistic) on rapid-signaling mechanisms that are normally regulated by endogenous estrogen. Thus, it is anticipated that phytoestrogens may modify the normal activities of endogenous estrogens during critical periods in the developing brain that may influence the function of the mature adult brain.

At the ER, the binding affinity and transactivational properties of different phytoestrogens appear dependent on the model system/cell type used for analysis and also vary for different orthologs of an ER in the same experimental system (Miksicek, 1994; Kuiper et al., 1997, 1998; Casanova et al., 1999; Matthews et al., 2000). As a result, it is difficult to directly compare the absolute binding affinities and transactivational potencies reported for different compounds across different systems. However, comparison of relative binding affinities from various studies indicates that some phytoestrogens appear to have a higher affinity for ER$_{\alpha}$ than for ER$_{\beta}$ and therefore suggests that the ER-mediated effects of phytoestrogens may be mediated through ER$_{\alpha}$ (Table 1). Because of the differential expression of ER$_{\alpha}$ and ER$_{\beta}$ in different tissues and cell types, this preferential binding of phytoestrogens at ER$_{\alpha}$ may in part explain the observed tissue-specific variability of phytoestrogen action.

As can also be seen from the results presented in Table 1, for all classes of phytoestrogens the binding affinity of a given phytoestrogen may not be predictive of its transactivational

### TABLE 1

Comparison of relative binding affinity and transactivational activity of phytoestrogens at human ER$_{\alpha}$ and ER$_{\beta}$

<table>
<thead>
<tr>
<th>Classification</th>
<th>Compounds</th>
<th>Binding Affinity</th>
<th>Transactivation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ER$_{\alpha}$</td>
<td>ER$_{\alpha}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ER$_{\beta}$</td>
<td>ER$_{\beta}$</td>
</tr>
<tr>
<td>Isoflavonoids</td>
<td>17$\beta$-estradiol</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Isoflavones</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Formononetin$^a$</td>
<td>&lt;0.01</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Daidzein$^a$</td>
<td>0.1</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Biochanin A$^a$</td>
<td>&lt;0.01</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Genistein$^a$</td>
<td>4</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>Ipriflavone$^a$</td>
<td>&lt;0.01</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Isoflavanones</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>O-Desmethylangolensin</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>Isoflavans</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>Equol</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Flavones</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apigenin$^a$</td>
<td>0.3</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Chrysin$^a$</td>
<td>&lt;0.01</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Flavone$^a$</td>
<td>&lt;0.01</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Flavonols</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kaempferol$^a$</td>
<td>0.1</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Quercetin$^a$</td>
<td>0.01</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Flavanones</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Naringenin$^a$</td>
<td>0.01</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Coumestrol$^a$</td>
<td>20</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Lignans</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Enterolactone</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>Enterodiol</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>Mycoestrogens</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zearalenone$^a$</td>
<td>7</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>$\alpha$-Zearalenol$^b$</td>
<td>48</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>$\beta$-Zearalenol$^c$</td>
<td>16</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>Zeranol</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., not determined.

$^a$ Kuiper et al., 1998.

$^b$ Matthews et al., 2000.

$^c$ Kuiper et al., 1997.
potency. In the case of the isoflavones, the relative binding affinity of daidzein at ERα and ERβ is 1000- and 200-fold lower than E2, respectively. However, the transactivational potency of daidzein (1000 nM) at an ER is nearly equivalent to that observed for the same concentration of E2 (Kuiper et al., 1998). In the case of genestin, its relative binding affinity is 25 times lower than that of E2 at ERα, whereas at ERβ the binding affinities of genestin and E2 are similar. In contrast to the observed differences in binding affinities, the transactivational potency of 1000 nM genistin at ERα and ERβ is about 2-fold greater than a similarly high concentration of E2 (Table 1) (Kuiper et al., 1998). The physiological relevance of the differences in ER-mediated transactivation potency at ERs that were detected at these supraphysiologica concentrations of E2 and phytoestrogens is unclear.

In the nervous system, prenatal and postnatal exposures to some environmental estrogens have been reported to result in long-term effects on neuroendocrine function and reproductive behavior (Palanza et al., 1999; Ferguson et al., 2000). Because of the lack of in vitro or in vivo data regarding the ability of phytoestrogens to influence rapid actions of E2 in the nervous system, the potential for these compounds to influence rapid E2-mediated mechanisms in the developing CNS is uncertain. However, it seems fairly likely that these compounds may also act as estrogen mimetics to influence some rapid E2-activated mechanisms, and based on the varying abilities of these compounds to bind ERs and to act as agonists of transactivation, it also seems plausible that phytoestrogens may influence rapid E2-mediated mechanism in a similarly variable and unpredictable fashion.

**Conclusion**

Recently, much new insight has accumulated concerning the mechanisms of estrogen action in reproductive and non-reproductive tissues. As a result, there has been increased understanding of, and appreciation for, the significance of both ER-dependent and -independent functions of estrogen in the CNS. Because both long-term genomic and rapid non-nongenomic E2-mediated mechanisms are likely to be important for normal development and function of the brain, there is a potential for environmental estrogens to impact brain functions and behavior by influencing these E2-mediated processes. In humans the available data do not confirm any risks associated with the exposure to normal dietary levels of phytoestrogens; however, much remains unknown concerning the risks associated with exposures to high concentrations of phytoestrogens. As a result, the influence of high concentrations of phytoestrogens during critical periods of neuronal development cannot be discounted completely. The uncertainties surrounding the actions of phytoestrogens on the CNS center around an incomplete understanding of the nongenomic role of E2 and its receptors in the brain, the variable ability of phytoestrogens to bind at and to act through ERs, and a nearly complete lack of experimental studies assessing the influence of environmental estrogens on the rapid actions of E2. The apparent importance of E2-mediated mechanisms during development and function of the CNS underscores the importance for detailed understanding of the mechanisms of E2 action in the brain. With a recent trend toward increased human exposures to higher concentrations of phytoestrogens, whether in diet or as an alternative to estrogen replacement therapy, additional research is needed to determine the effects that phytoestrogens may have on the developing and mature nervous system.

**References**


Address correspondence to: Scott M. Belcher, Ph.D., Department of Pharmacology and Cell Biophysics, University of Cincinnati College of Medicine, P.O. Box 670575, Cincinnati, OH 45267-0575.