Similarities between actions of estrogen and BDNF in the hippocampus: coincidence or clue?

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The principal ovarian estrogen, estradiol, and brain-derived neurotrophic factor (BDNF) have widespread effects on the CNS that have usually been studied independently. This article examines the similarities in the effects of estradiol and BDNF in the hippocampus, in light of the evidence that estradiol can induce BDNF expression, and recent data suggesting that structural and electrophysiological effects of estradiol in the hippocampus might be mediated by BDNF. The possible role of BDNF as a signaling molecule downstream of estrogen in the hippocampus has implications for our understanding of several cellular and behavioral hippocampal functions, including dendritic and synaptic plasticity, learning and cognitive behavior. Furthermore, disruption of the relationship between estrogen and BDNF could contribute to neurological and psychiatric disorders that have been associated with the hippocampus, such as Alzheimer's disease, depression and epilepsy.

Introduction

Estrogens have profound effects on the structure and function of the hippocampus [1,2]. These effects are thought to be mediated primarily by nuclear estrogen receptors (ER), ERα and ERβ, that interact with components of the chromatin to regulate transcription of estrogen target genes [3]. In addition, estrogens might also act on cells by interacting with ER-like proteins associated with the plasma membrane [4]. Such membrane receptors appear to exist in the hippocampus, where they could be involved in rapid or highly localized nongenomic actions of estradiol on hippocampal function [5]. Therefore, the mechanisms responsible for translation of ER-activated cellular responses into the diverse structural and physiological changes observed in the hippocampus following exposure to estrogen remain uncertain. In this article, we examine the evidence that estrogen could act on the hippocampus, at least in part, using an intermediate signaling molecule, brain-derived neurotrophic factor (BDNF). We suggest that electrophysiological and behavioral, as well as trophic, effects of estrogen in the hippocampus could be mediated by regulation of BDNF, ultimately relying on signal transduction pathways activated by neurotrophin receptors.

Historical background

Studies relating the actions of steroid hormones to those of growth factors have a rich history, originating in research on the mechanism of estrogen action in non-neural target organs. In the breast and female reproductive tract, the transcriptional effects of estrogen include increased synthesis of growth factors [6,7], leading to autocrine and paracrine actions that last for some time after initial estrogen receptor occupation has declined [8]. Thus, epidermal growth factor (EGF) and insulin like growth factor-1 (IGF-1), whose synthesis is stimulated by estradiol in breast cancer cells, activate ER phosphorylation, thereby enhancing the rate of transcription of estrogen-responsive genes [9].

The involvement of growth factors in the responses of the brain to estrogen was first suggested by observations of synergism between the effects of estrogen and IGF-1 in the developing brain [10], and by the discovery that estrogen-sensitive neurons often express neurotrophin mRNA [11]. Subsequently, a sequence was identified on the BDNF gene that was similar to the estrogen-sensitive response element (ERE) found in other estrogen-regulated genes [12], consistent with the idea that estrogen might regulate BDNF synthesis. Studies in female rat hippocampus showed that ovariectomy decreased levels of BDNF mRNA, and that estradiol treatment could reverse the effect [12–14]. Subsequent studies demonstrated that administration of estradiol to gonadectomized male rat pups restored BDNF expression in hippocampal pyramidal cells; an ERE mechanism was implicated because ERα colocalized with BDNF [15]. Taken together, these studies suggested that the actions of estradiol in the hippocampus might include regulation of BDNF.

Similarities between the actions of estrogen BDNF

Although consistent with the trophic effects of estrogen in the developing brain, the studies described in the preceding paragraphs did not establish whether the induction of BDNF by estrogen might influence hippocampal function. A large literature now suggests that this might, in fact, be the case.

The first line of evidence consists of correlations between the effects of estrogen and BDNF. Estradiol and BDNF have similar actions, and the range of these actions is both extensive and complex. The similarities include enhancement of glutamate-mediated transmission

in principal cells [2,16,17], modulation of NMDA receptors and specifically the NR2B subunit [18,19], decreased afterhyperpolarizations (in CA1 neurons) [20–22], facilitation of memory [1,18,23,24], increased dendritic spine and spine synapse numbers [5,23,25], and promotion of dentate gyrus neurogenesis [26–28]. In addition, estrogen and BDNF can increase seizure susceptibility [29,30], although there are exceptions (as will be discussed later). Similarly, estrogen and BDNF have each been implicated in neuroprotection after ischemia, but there are also reports, again for both estrogen and BDNF, that damage can be exacerbated [31–33]. To our knowledge, no other two compounds share this diverse and complex spectrum of effects, suggesting that the similarities might not be coincidental.

A second correlation is that the signal transduction pathways and transcription factors implicated in the effects of estrogen and BDNF also appear to be shared. These include the p42/44 mitogen-activated protein kinase (MAPK) pathway [also known as the extracellular-regulated protein kinase (ERK) cascade] [18,34,35], the phosphatidylinositol 3-kinase (PI3-K) pathway [36–38], Src/Fyn [18,35], Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) [39,40], and cAMP-response-element-binding protein (CREB) [41,42].

As already mentioned, effects of estrogen and BDNF on seizures and neuronal damage appear to be complex. The fact that the same compounds can be both neuroprotective and exacerbate damage, for example, suggests that estradiol and BDNF probably influence multiple variables that can interact to produce varying net effects. One example is neuropeptide Y, which is induced by activity and by BDNF [43] and has potent actions in the hippocampus, including anticonvulsant and neuroprotective effects [44]. Neuropeptide Y can be induced in GABAergic neurons or principal cells, or both, and induction can be either transient or long lasting [43,44], and hence can have variable consequences. Other possible variables also complicate effects of estrogen and BDNF in the hippocampus. For example, estrogen and BDNF both interact with 5-hydroxytryptamine (5-HT) [45] and IGF-1 [46], factors with powerful effects on hippocampal structure and function.

Functional evidence for the hypothesis that estradiol acts via BDNF

The first evidence that BDNF mediates effects of estradiol in the hippocampus came from cultured developing hippocampal neurons [25]. Estradiol increased dendritic spine number, via a mechanism dependent on BDNF. However, estradiol decreased BDNF levels – opposite to what one would predict from previous data from intact adult rats [13,15]. This decrease could be due to the proposed role of GABA-mediated inhibition in the effects on spines [25]; indeed, a similar hypothesis has been proposed in the adult [2]. However, in development, GABAergic neurons could have effects that are distinct from those in the mature brain, because GABA has trophic effects and is depolarizing at certain times in development, but this changes with maturity [47]. The ways that estradiol and BDNF interact might also be highly dependent on developmental stage, given the fact that hippocampal concentrations of ER change dramatically during the course of postnatal life [48].

We have examined this issue from a different approach, based on previous studies conducted in hippocampal slices from male rats and mice. Initial studies showed that exposure of male rat hippocampal slices to recombinant BDNF, or stimulation of slices from transgenic male mice overexpressing BDNF, led to a stereotypical effect on transmission from the mossy fiber axons of dentate granule cells to area CA3 pyramidal cells; this effect could be blocked by an antagonist of trk receptors, which bind neurotrophins [49,50] (Figure 1). Thus, trains of population spikes were evoked by stimulation, and only brief low-frequency (1 Hz) stimulation of the mossy fibers was required. The effects could not be reproduced by stimulation of other CA3 inputs, even at maximal intensities. Subsequent studies showed that other drugs that produce epileptiform activity, such as disinhibitory agents, did not reproduce this effect, making it seem unique. As such, it appeared useful to probe the potential influence of estrogen on BDNF-dependent changes in mossy fiber transmission. Furthermore, because the highest concentration of BDNF protein in hippocampus is in the mossy fiber pathway [51], this approach potentially provided insight into the role of endogenous hippocampal BDNF.

Remarkably, in hippocampal slices from intact female rats that were sampled during the morning surge of estradiol (at proestrus), there was a change in mossy fiber transmission that mimicked slices from male rats exposed to BDNF, or male mice overexpressing BDNF (Figure 1). BDNF immunocytochemistry confirmed that mossy fiber BDNF expression increased at proestrus, and indeed remained elevated for ~24 h (Figure 2). An antagonist of trk receptors reversed the effect [52]. These data are consistent with the hypothesis that the effects of estradiol on mossy fiber transmission at proestrus could be mediated by upregulation of BDNF.

Critical evaluation of the hypothesis

Although this hypothesis is potentially attractive because it could provide greater insight into the roles of estrogen and neurotrophins in the hippocampus, several studies indicate that there is unlikely to be a simple relationship between estrogen and BDNF. We will now discuss three examples of the complications raised by past studies.

First, estradiol and BDNF can induce different effects in the hippocampus even when the same endpoint is studied, such as seizure susceptibility or neuroprotection (as already discussed). Many of these discrepancies can be explained by differences in experimental conditions (e.g. the use of different ages, genders or doses, and the comparison of intact with ovariectomized females). However, it is also likely that variables that are not yet fully understood, such as levels of pro-neurotrophins [53], could vary with experimental preparation.

A second issue is that changes in BDNF immunoreactivity during the estrous cycle are not necessarily consistent with studies of BDNF mRNA expression. During the estrous cycle, BDNF mRNA levels

are highest in the hippocampus at diestrus [54], when levels of both BDNF protein and circulating estradiol are low. The same study showed that mRNA levels were lowest during the afternoon of proestrus relative to other cycle stages that were sampled. A separate study likewise demonstrated that levels of BDNF mRNA in the dentate gyrus were lower at proestrus than at estrus [55]. Although these studies are consistent with the data of Murphy and Segal on cultured hippocampal neurons [25], they are discordant with both our data (Figure 2) and data from ovariectomized animals showing induction of BDNF mRNA after estrogen administration [12–14]. Part of this discordance could be due to the fact that time of examination is important in intact cycling females. For example, in the study by Gibbs [53], changes in the expression of BDNF mRNA in the dentate gyrus and CA1 were larger in the afternoon of proestrus, when hormones other than estradiol (particularly progesterone) could also affect hippocampal function. By contrast, our immunocytochemical studies [51] used animals on the morning of proestrus, before the ovulatory rise in ovarian progesterone secretion has begun [56]. In addition, BDNF mRNA levels are regulated not only by estrogen but also by neuronal activity [57], corticosteroids [58] (levels of which rise during proestrus) and age [59]. Furthermore, BDNF synthesis can be regulated not only at the transcriptional level but also at the level of translation. Both estrogen [60,61] and BDNF itself [62] influence translation in hippocampal dendrites and synapses. Finally, it should be noted that non-neuronal cells can also make a significant contribution to BDNF mRNA levels. Glia can both synthesize and respond to BDNF, and have been hypothesized to play a crucial role in estrogen-induced hippocampal remodeling [63,64].

The complex nature of the mechanisms regulating BDNF synthesis could help us to understand the third problem in the literature: that there appears to be a 'mismatch' between the distributions of ER-expressing and BDNF-expressing cells in the hippocampus. Although, as previously mentioned, colocalization of BDNF and ERα has been reported in developing rat brain [15], during early development the rodent hippocampus has a far higher level of ER expression than in adulthood [48]. In the adult, little identifiable ERα or ERβ immunoreactivity is present in BDNF-immunoreactive hippocampal neurons [65–67], although this is controversial [68–70]. Physiological state could be an important variable, because most studies have failed to detect significant granule cell ER expression, but ERα has been detected in granule cells of proestrous female rats [68]. Analogous issues arise in discussion of BDNF localization: although BDNF mRNA is clearly present in each of the principal cell populations of the hippocampus (i.e. granule cells, and CA1 and CA3 pyramidal cells), one antibody shows primarily mossy fiber expression with little pyramidal cell protein content [51] (Figure 2) and another shows primarily somatic and dendritic protein expression [15,71].

If there are no estrogen receptors on BDNF-expressing neurons such as the granule cells, estrogen could still regulate BDNF synthesis without invoking an ERE mechanism. Such regulation might occur indirectly, via estrogen-sensitive inhibitory interneurons [65]. This line of reasoning is supported by the demonstration that ER are localized in GABAergic neurons throughout hippocampus [66,67], that estrogen appears to depress inhibition in the hippocampus [2] and that BDNF synthesis is activity-dependent [57]. Thus, estrogen-mediated inhibition of GABAergic activity could secondarily increase activity in the granule cells, thereby indirectly increasing BDNF synthesis. The single argument against this hypothesis is the report that BDNF can exert its effects on dendritic spines in the absence of synaptic transmission, although synaptic transmission can modulate the phenomenon [72].

Given these considerations, Figure 3 shows possible mechanisms that could be responsible for the effect of estradiol on mossy fiber BDNF that would not require an ERE mechanism. First, there could be an indirect effect mediated by disinhibition (Figure 3a). Second, there could be a localized influence on BDNF synthesis mediated by membrane ER (Figure 3b). It is important to add that an ERE-related mechanism cannot yet be ruled out entirely, because of the current controversy surrounding ER expression.

Implications for CNS function and dysfunction

Despite the complexities that remain to be resolved, the potential for interaction between BDNF and estrogen has some important implications for both normal and abnormal hippocampal function. Thus, under normal conditions, the effects of estrogen on synaptic structure and function, and on learning and memory, could be mediated at least in part by BDNF. Conversely, disruption of estrogen–BDNF interactions could lead to disorders that have been related to hippocampal functions. For example, in Alzheimer's disease, estrogen might ameliorate symptoms in part because of its ability to upregulate BDNF. In fact, expression of BDNF is reduced in the hippocampus and parietal cortex in Alzheimer's disease [59]. Although a recent large-scale clinical trial of hormone replacement failed to demonstrate significant protection against cognitive impairment in postmenopausal women aged ≥65 [73], an important feature of this trial is that it utilized long-term continuous treatment. Laboratory studies suggest that the inductive effects of estrogen on BDNF levels are not maintained during long-term estradiol therapy [55].

Depression is another disorder that might involve disruption of estrogen–BDNF interactions in the hippocampus, because dentate gyrus neurogenesis has been linked to depression [74], and both estradiol and BDNF positively modulate granule cell neurogenesis. A role for estradiol in depression is also suggested by the facts that depression is more common in women than in men, that depression increases after menopause, and that estradiol can alleviate symptoms or enhance antidepressant action [75,76]. Analogous to the situation in Alzheimer's disease already discussed, BDNF deficits occur in depressed individuals and have been implicated mechanistically [77]. Thus, inadequate stimulation of

dentate gyrus BDNF synthesis by estradiol could contribute to depressive symptomatology.

Another clinical condition that could be related to estrogen–BDNF interactions is epilepsy. In women with seizures that involve the hippocampus (temporal lobe epilepsy), and likewise in a subset of women with catamenial epilepsy, seizure frequency increases at ovulation [78,79]. Increased seizure frequency during the periovulatory period is exactly what one would predict based on data from rodents (Figures 1 and 2) because that appears to be when estradiol–BDNF interactions are initiated. However, much more research will be needed before these hypotheses can be translated into new clinical strategies.

What about the male?

Although the discussion here has focused on females, estrogen is just as relevant for males because intracerebral estrogen biosynthesis has an important role in mediating the central effects of circulating androgens [80]. Testosterone is aromatized to estradiol in the hippocampus [81], where it contributes to synaptic remodeling and neuronal survival [82]. There is considerable evidence indicating that low circulating testosterone levels could be associated with an increased risk of Alzheimer's disease in men [83]. Serum estrogen concentrations often rise in men with epilepsy, and it has been proposed that this contributes to their seizures [84–86]. Thus, the potential clearly exists for estrogen-mediated regulation of BDNF expression to contribute to hippocampal responses in males.

Concluding remarks

There is now substantial evidence in both the periphery and the brain that interactions between steroid hormones and growth factors are important. In the hippocampus, the link between estrogen and BDNF appears to be a prime example. Estrogen-mediated regulation of BDNF could involve several component mechanisms: direct regulation of BDNF gene expression involving nuclear ERα and/or ERβ; membrane receptors that lead to BDNF synthesis at extranuclear sites; indirect effects mediated via estrogen action on GABAergic interneurons; interactions with other steroid hormones; and possible effects on glia. Actually, these various regulatory components might all have important roles, depending on age or endocrine status. In summary, the control that estrogen can potentially exert over BDNF synthesis could have important consequences for hippocampal function, as well as dysfunction, and this could be the case in both sexes.

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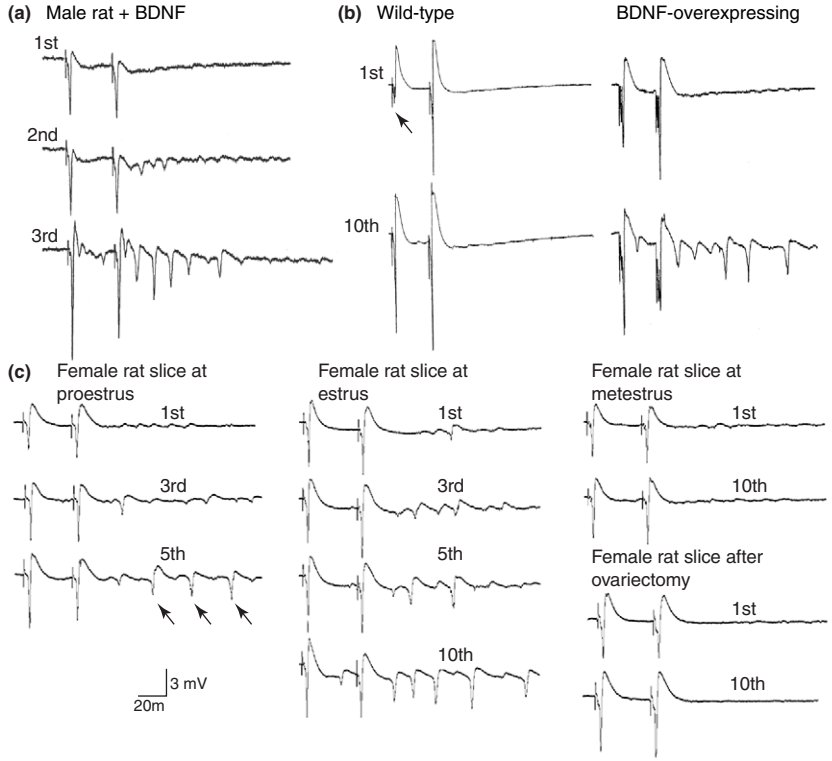

Figure 1. Similar effects of BDNF on mossy fiber transmission under different experimental conditions. **(a)** Exposure of a male rat hippocampal slice to recombinant BDNF led to abnormal excitability: in response to three pairs of successive mossy fiber stimuli (numbered 1st, 2nd and 3rd in the figure), multiple population spikes developed (arrows). This effect was absent in slices exposed to vehicle. In these experiments, stimulus strength was set to half-maximal, interstimulus interval was 40 ms and stimulus pairs were triggered at 1 Hz. Reprinted, with permission, from Ref. [49] © (1997) The American Physiological Society. **(b)** In response to the tenth stimulus pair to the mossy fibers [using the same stimulus paradigm as in (a)], multiple population spikes were recorded from the area CA3 cell layer of a hippocampal slice from a BDNF-overexpressing mouse (right) but not in a slice from a wild-type mouse (left). Note that endogenous mossy fiber BDNF levels were elevated in the BDNF-overexpressing mice but not in the wild-type mice. Reprinted, with permission, from Ref. [50]. **(c)** Responses to successive pairs of mossy fiber stimuli [using the same stimulus paradigm as in (a)] are shown for hippocampal slices from four different female rats. After the first stimulus pair, multiple population spikes developed in the area CA3 cell layer of the hippocampal slices from proestrous and estrous rats (responses to the 1st, 3rd, 5th and 10th stimulus pairs are shown) but not in those from metestrous or ovariectomized rats (responses to the 1st and 10th stimulus pairs are shown). Recombinant BDNF exposure was not required for this effect, presumably because endogenous mossy fiber BDNF levels were elevated at proestrus and estrus. Reprinted, with permission, from Ref. [52] © (2003) Society for Neuroscience.

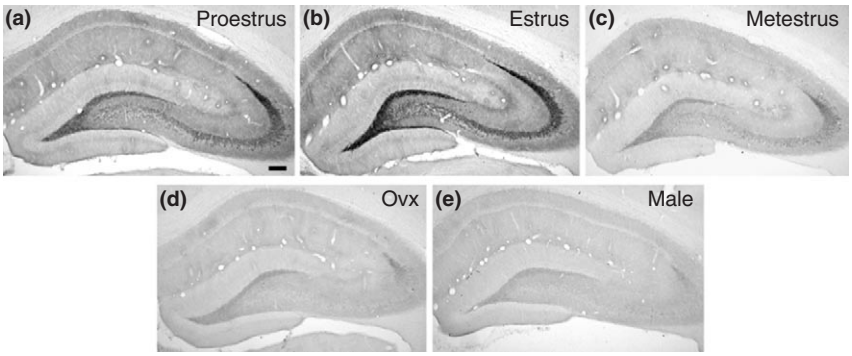

Figure 2. Variation in BDNF expression during the estrous cycle of the rat. A comparison of BDNF protein expression, using sections processed concurrently, from female cycling rats in proestrus **(a)**, estrus **(b)** and metestrus **(c)**, from an ovariectomized (ovx) rat **(d)** and from a male rat **(e)**. Note that male rat BDNF levels can appear much greater than shown if incubation time is extended [51]. However, incubation was abbreviated to allow differences among experimental groups to be most easily appreciated. Reprinted, with permission, from Ref. [52] © (2003) Society for Neuroscience.

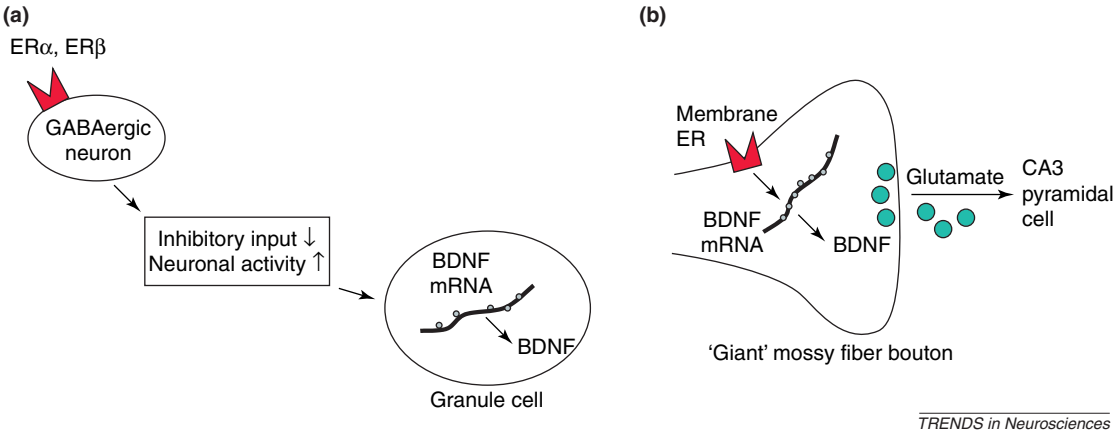

Figure 3. Ways in which mossy fiber BDNF expression might be regulated by estradiol without invoking classic nuclear ER. **(a)** Ligand-bound ER on GABAergic interneurons depress inhibitory input to granule cells, increasing activity of the granule cells and consequently activating BDNF synthesis. **(b)** Ligand-bound membrane ER on 'giant' mossy fiber boutons activate BDNF synthesis in the terminal. Although local protein synthesis has primarily been described in hippocampal dendrites rather than synaptic terminals [62], the unique and sophisticated nature of the massive mossy fiber terminal [87] and new consideration of axonal translation [87], particularly in axons that can grow in the same way as mossy fibers [87], support this hypothesis. Note that BDNF is likely to be compartmentalized to the massive boutons of the mossy fibers, not to the relatively small boutons that are thought to innervate interneurons primarily [89,90]. Such localization presumably allows preferential increase in glutamate release onto CA3 pyramidal cells, and the consequent increase in excitability (Figure 1), without a concurrent increase in inhibition that would dampen excitability.