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The dual action of estrogen hypothesis

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Estradiol (E_2) can act in the brain in a relatively fast manner (i.e., seconds to minutes) usually through signaling initiated at the cell membrane. Brain-derived E_2 has thus been considered as another type of neurotransmitter. Recent work found that behaviors indicative of male sexual motivation are activated by estrogenic metabolites of testosterone (T) in a fast manner, while sexual performance (copulatory behavior *per se*) is regulated by brain E_2 in a slower manner via nucleus-initiated actions. This functional division between these two types of action appears to generalize to other behavioral systems regulated by E_2. We propose the dual action of estrogen hypothesis to explain this functional distinction between these two different modes of action.

Neuroestrogens versus ovarian estrogens

Estrogens are steroid hormones that were initially isolated and characterized in the late 1920s by Adolf Butenandt (Nobel laureate for this discovery) and Edward A. Doisy [1]. They are present in all vertebrates and some invertebrates, and are traditionally considered to be the primary female sex hormone because they are produced by the ovary, released into the blood, and play a key role in the control of female reproduction. Estrogens are secreted in the developing ovarian follicles by aromatization of androgenic substrates that have been produced locally. Aromatization is a complex enzymatic reaction catalyzed by aromatase (also known as estrogen synthase or EC 1.14.14.1, the product of gene *CYP19*), an enzyme of the cytochrome P450 family. Aromatization of androgens includes three successive hydroxylations resulting in the elimination of one methyl group and acquisition of a fully saturated (aromatic) 'A' ring [2]. The main substrates of aromatization are the androgens T and androstenedione that are transformed by aromatase into 17β-estradiol (E_2) and estrone (E_1), respectively.

In the late 1960s a marked level of aromatase activity (AA) was discovered in the brain of mammalian species including humans [3], and this finding was soon generalized to representative species in the major vertebrate classes (i.e., fish, reptiles, birds [4]). These claims were based on data from radioenzyme assays quantifying the transformation of radioactive T into radioactive E_2 that were later supplemented by the identification of the presence of the relevant mRNA (by PCR or *in situ* hybridization) and of the enzymatic protein (by immunohistochemistry or western blot). This finding of widespread brain aromatase opened an entirely new field of investigation that resulted over the years in the demonstration that estrogens produced in the brain (termed neuroestrogens), either by the aromatization of testicular T or via a series of enzymatic steps from the precursor of all steroids, cholesterol [5,6], play a decisive role in (i) the sexual differentiation of brain and behavior, and (ii) the activation of male sexual behavior in rodents and many other vertebrate species [7]. Thus the notion that estrogens should be considered a 'female' hormone is an over-simplification. Estrogens have important roles in males as well. During the past few decades it was discovered that estrogens additionally control a host of physiological and pathological traits ranging from bone formation, lipid metabolism, and brain plasticity and repair to tumor growth. They are now considered as pleiotropic signaling factors whose action extends well beyond the control of reproduction.

The dual control of estrogen synthesis

Ovarian and brain estrogens have exactly the same chemical structure and are produced by the same enzyme, aromatase. Two fundamentally different controls of brain AA have been identified that operate in distinct time-domains. In the long term, steroids increase transcription of the aromatase gene, resulting in increased enzyme concentration and thus activity (genomic control). In a shorter timescale, post-translational modifications of the enzymatic protein drastically modify its activity within minutes without changing its concentration (post-translational control) (Box 1).

Fast versus slow actions of estrogens

As other steroid hormones, estrogens are classically considered to exert their biological effects by binding to their cognate nuclear receptors [i.e., estrogen receptor α (ERα) and estrogen receptor β (ERβ)], which then act as ligand-activated transcription factors to regulate transcription of specific genes associated with an estrogen-responsive element (ERE) or other types of response elements (e.g., AP1, Sp1, NF-κB) [8]. These biological effects are thus mediated via transcription of genes, translation of corresponding mRNAs into proteins, and their integration into functional pathways; they take hours to days to develop.

As early as 1976 it was observed that in the brain estrogens exert, in addition to these relatively slow effects, electrophysiological effects with latencies of only a few seconds [9]. This discovery was at the origin of an entirely

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Box 1. Two independent controls of aromatase activity

The genomic controls of aromatase synthesis are very different from one tissue to another. The human aromatase gene is composed of nine coding exons and multiple alternative non-coding first exons that regulate this tissue-specific expression [2,58]. A similar situation has been identified in other species including rodents [58]. Depending on which of the alternative untranslated first exons is used for transcription, aromatase can be upregulated by androgens (brain), cAMP (ovary, testis, placenta), dexamethasone (liver, adipose and vascular tissue, skin), or retinoic acid (bone), among others [58]. Aromatase activity (AA) and estrogen production are consequently modified in parallel with changes in aromatase concentration. In the brain for example, AA increases after a few hours to a few days following exposure to T [59,60].

An alternative mechanism of AA control was identified more recently that does not involve changes in enzyme concentration and takes place more rapidly (5–15 min). We serendipitously discovered that AA in quail (*Coturnix japonica*) brain homogenates is rapidly (within minutes) inactivated by phosphorylating conditions (high but physiological concentrations of ATP, Mg^{2+}, and Ca^{2+} [61,62]), an effect that is blocked in the presence of kinase inhibitors. Subsequent investigations performed on preoptic-hypothalamic explants maintained *in vitro* indicated a similar rapid inactivation of the enzyme activity by phosphorylation that is itself under the control of glutamatergic transmission [63]. This phosphorylation-dependent inactivation of AA was also demonstrated in various cell lines transfected with the human aromatase gene as well as in quail ovaries [64]. Work conducted in the zebra finch (*Taeniopygia guttata*) telencephalon indicated that this acute modulation of AA by phosphorylating conditions occurs specifically at the level of synaptic terminals but not in the perikaryon [65]. *In vivo* dialysis in the auditory cortex of the same species provided converging evidence that glutamate rapidly decreases local estrogen concentrations [66], and that this effect results from changes occurring at the level of synapses [67]. Social interactions with a congener of the opposite sex and acute stress also modulate, within minutes, brain AA and/or local estrogen concentration in a sex- and anatomically-specific manner [68–71].

new line of research that resulted in the identification of a second mode of estrogen action usually initiated at the cell membrane and that is associated with shorter latencies in the second to minute range. These rapid membrane-initiated effects of E_2 are mimicked by E_2 associated with a larger molecule that prevents its entry into the cells [e.g., E_2 conjugated to bovine serum albumin (E_2–BSA) or E_2–biotin] [10]. They are mediated by the activation of intracellular signaling cascades resulting in changes in intracellular calcium concentration or, in a slightly slower manner, in the phosphorylation of various proteins including transcription factors such as the cAMP response element-binding protein (CREB), inducing in this way the so-called indirect genomic effects that appear with longer latencies than real non-genomic effects [11].

More recently, research has also implicated at the organismal level this mode of estrogen action in the control of a variety of behaviors including male and female sexual behavior, aggression, nociception, learning and memory, and auditory signal processing (see [12,13] for review). The first of these behavioral studies in the context of sexual behavior reported that a bolus of E_2 activates within 35 min anogenital sniffing and mounting in castrated male rats, *Rattus norvegicus* [14]. Subsequent studies extended this notion of rapid effects of E_2 on sexual behavior to Japanese quail (*Coturnix japonica*) and mice (*Mus musculus*) by showing that a single injection of the aromatase inhibitor rapidly (within 10–30 min) and transiently reduces various components of male sexual behavior [15,16]. Additional experiments also demonstrated that, in these two species, a single E_2 injection increases male sexual activity within 10–15 min [16,17].

The experiments in quail had two important features in common. First, the rapid effects of estrogens on sexual behavior were easier to demonstrate when males were in addition exposed to a weak stimulation by androgens (a low dose of T [17]). Second, there was a clear suggestion that these rapid effects concerned mostly the initial phases of the interaction with the females rather than the copulatory performance *per se*. In the study assessing rapid behavioral effects of aromatase inhibition, two aspects of appetitive sexual behavior (ASB) – the rhythmic contractions of the cloacal sphincter muscles (RCSM) and the learned social proximity response (LSPR) in a two-compartment chamber (behaviors described in Box 2) – were for example more reliably inhibited (effect sizes 0.408 and 0.534, respectively) than the copulatory act *per se* (effect sizes 0.144 or 0.251 for the low and high doses of inhibitor, respectively) [15].

Distinct controls of behavioral motivation and performance

This distinction was confirmed and reinforced by more recent experiments during which brain estrogen bioavailability was pharmacologically manipulated by intracerebroventricular (ICV) injections. For these studies, castrated male quail that were chronically treated with exogenous T (subcutaneous Silastic™ implant) were implanted with an ICV cannula in the third ventricle, and then were used as their own controls in successive behavior trials after injections of aromatase inhibitors or various forms of estrogens [18].

The frequency of RCSM was reduced by more than 50%, 30 min after a single ICV injections of two anti-estrogens, either tamoxifen or ICI182,780 (i.e., Fulvestrant or Faslodex™), as well as of Vorozole™ or ATD (1,4,6-androstatriene-3, 17-dione), two aromatase inhibitors (Figure 1A,B). A very significant inhibition of RCSM was already observed 15 min after injection of tamoxifen or ICI182,780. By contrast, the frequency of cloacal contact movements (CCM), the actual copulatory pattern (Box 2), was completely unaffected by all these manipulations (Figure 1C,D).

The behavioral inhibitions observed after injection of aromatase inhibitors were clearly related to the depletion of local estrogen synthesis because they could be prevented by a single injection of exogenous E_2 (50 μg/bird 15 min before testing; i.e., 15 min after injection of the aromatase inhibitor; Figure 2A). The short latency of this behavioral effect suggested that it did not rely on new protein synthesis, and was thus mediated by non-genomic mechanisms; this was confirmed by the fact that the Vorozole-induced inhibition of RCSM could similarly be prevented by an acute injection of E_2–biotin (Figure 2B).

The blockade of RCSM, but not of copulation, following inhibition of neuroestrogen production or action suggested dissociation between mechanisms controlling sexual motivation and performance. To test whether this distinction could be generalized, we assessed the effects of these