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Review

Estradiol's interesting life at the cell's plasma membrane

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ABSTRACT

Clearly, we have presented here evidence of a very complex set of mechanisms and proteins involved with various and intricate actions of steroids at the plasma membrane. Steroids do MUCH more at the plasma membrane than simply passing passively through it. They may sit in the membrane; they are bound by numerous proteins in the membrane, including ERs, SHBG, steroid-binding globulin receptors, and perhaps elements of cellular architecture such as tubulin. It also seems likely that the membrane itself responds graphically to the presence of steroids by actually changing its shape as well, perhaps, as accumulating steroids. Clara Szego suggested in the 1980s that actions of E2 at one level would act synergistically with its actions at another level (e.g. membrane actions would complement nuclear actions). Given the sheer number of proteins involved in steroid actions, just at the membrane level, it seems unlikely that every action of a steroid on every potential protein effector will act to the same end. It seems more likely that these multiple effects and sites of effect of steroids contribute to the confusion that exists as to what actions steroids always have. For example, there is confusion with regard to synthetic agents (SERMs etc.) that have different and often opposite actions depending on which organ they act upon. A better understanding of the basic actions of steroids should aid in understanding the variability of their clinical effects.

Contents

Abbreviations: CBG, corticosteroid binding globulin; CHO cell, Chinese hamster ovary cell; CNS, central nervous system; E2, estradiol; ERα, estrogen receptor alpha; ERβ, estrogen receptor-beta; Erk-1/2, extracellular-signal regulated kinase; GC, glucocorticoid; GPR30, G-protein coupled receptor 30; RBP, retinol binding protein; SHBG, sex hormone binding globulin; Stra6, stimulated by retinoic acid 6 transmembrane protein.

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The key ovarian steroid in humans, estradiol, is perhaps the most potent single molecule in the human body. It demonstrates a plethora of physiological effects on almost every organ in the body and it seems to have more pathways of physiological action than any other molecule. We are still discovering whole avenues of its effects. Many physicians and endocrinologists devote any attention that they pay to estradiol entirely to its manifold effects in the cell's nucleus. There is a pharmacology review for preparing students for their medical exams that has this line, "Mechanism of action utilizes intracellular receptors" as the only thing medical students need to know about steroids. In contrast, this review will focus on some little noted, but still extremely fascinating, effects of estradiol on the cell's plasma membrane envelope and will suggest there are effects of the membrane back on estradiol. In short, it will review the very interesting life of estradiol at the cell's plasma membrane.

1. Well, it's in all of the textbooks

As indicated above, the currently taught version of how all steroids work is dictated by what endocrinologists call the "Free Steroid Hypothesis". Briefly, this hypothesis states that steroids are carried in the blood by proteins known as steroid-binding globulins. Around their target organs, these steroids exist in equilibrium with their binding proteins and as in any system in equilibrium, if the free moiety is being drawn down, by for example diffusion into another compartment, more of the bound element is released into the free reservoir. This free moiety of steroids, since everyone knows that steroids are lipophilic, passes freely through the plasma lipid layer of the cell membrane and into the cytosol where it is grabbed by important cytosolic receptors. A number of events may occur at this point, but basically the steroid with its receptor passes into the cell nucleus where it has many, many effects on transcription of genes. In other reviews [1–3] we have argued against the viability of the Free Steroid Hypothesis, and we will not reiterate in this paper either the cogent arguments of numerous researchers or the chronology of our own path to believing that there are fallacies in the Free Steroid Hypothesis.

There may be many places where this model does not work very well, but the most jarringly illogical point is on the inside of the plasma membrane. What could possibly induce a steroid, sitting snuggly in its lipid bilayer, to come out of that layer and jump into the aqueous cytosolic environment? The answer to that question is—nothing. No self-respecting steroid would leave the lipid bilayer, without some help, to go into the water inside the cell. Allera and Wildt [4] clearly demonstrated that without some protein element, steroids did not even pass through the lipid bilayer. So, if steroids do not just pass through the cell membrane, what happens to them there? This question is the basis of this entire paper. What happens to the steroid at the level of the cell's plasma membrane envelope?

2. Estradiol is being grabbed

One possibility as to what is happening to the ovarian steroid estradiol (E2) at the membrane is that, unlike what is suggested in the Free Steroid Hypothesis, it is grabbed by a membrane-associated receptor. That is the reason that there need to be proteins in the plasma membrane for E2 to move through the membrane. This could be a membrane-associated receptor for E2. Many excellent laboratories have been engaged in trying to determine what this receptor or these receptors are.

One candidate for a grabber of E2 in the cell's membrane is GPR30. In the 1990s it was found that E2 stimulated cellular functions via second messengers Erk-1 and Erk-2 [5]. This study demonstrated that this effect could be seen in a cell line that did not express either estradiol receptor alpha (ERα) or ERβ suggesting that the membrane-associated GPR30 receptor not only mediates rapid actions of E2, but that it does so without the assistance of either of the intracellular ERs α or β. The possibility that many of the proteins involved in binding and carrying steroids such as E2 interact to mediate even a single action of a steroid is a relevant consideration and some models of such combined and synergistic action will be presented later in this review. Actions of E2 via GPR30 have been demonstrated in cell lines linked to breast cancer [6] and thyroid cancer [7]. Oddly, however, even though there are antibodies that apparently have specificity for GPR30 at least in blot material [8], there has been no attempt to map the localization of GPR30 in a range of organs. Interestingly, with regard to the issue of interactions among various grabbers of steroids is evidence that GPR30 and ERβ may be found in the same cells [9] where they may interact to mediate E2's effects.

Martin Kelly has proposed a separate membrane-associated estradiol receptor [10–17]. Their ER has rapid effects that are mediated both by interaction with GABA receptors [13,18] and associated with opiate neurons [18]. They have found a synthetic agonist that is specific to their ER called ST-X [15,19]. Unlike GPR30, Kelly has not yet presented evidence that their ER is associated with other known response factors for E2.

3. What about the inside of the plasma membrane?

It should be mentioned that it is controversial whether GPR30 even has a role in E2 actions. Kang et al. [20] claim that many of the actions attributed to GPR30 are actually mediated by a splice variant of ERα called ERalpha-36. Ellis Levin is a prominent researcher in the field and he has claimed that ERα is selectively moved to the inside of the plasma membrane [21–23]. They have even defined the elements that are required to direct ERα to the inner side of the plasma membrane. In spite of this, Levin does not claim that ERα binds extracellular E2 from this position.

This raises the obvious question, if cells go through all of the energy expenditure to shunt ERs to the membrane, what are they doing there? Although Levin's laboratory does not claim that ERs bind extracellular E2, other laboratories do. Mermelstein et al. [24,25] have demonstrated that ERα is shunted to the cell's membrane where it interacts with glutamate receptors to influence cell excitability. A process known as palmitoylation is essential for this to occur [26]. The Micevych laboratory has also studied the role of ERα at the membrane as well, which they suggest is sometimes mediated by a splice variant of ERα [27]. Their laboratory has done extensive work to examine the role of membrane ERs interacting with the brain's opiate system [28–30], which they link to central control of female sexual receptivity [30,31].

Another possibility suggested by the presence of ERs on the inside of the plasma membrane, but not having a function to bind extracellular E2, is that they are there as part of a larger complex of binding proteins and response proteins that either help to internalize E2 or help to mediate some function of E2 at the plasma membrane. This idea will be further explored below.

4. A collection of proteins in caveolae

Several of the studies cited above suggest that ERs are shunted to the level of the cell membrane, from which site they may function as part of a complex involved in either E2 uptake or intracellular responses to E2. Could the cell membrane really have so many proteins just involved in uptake of E2? The answer is "yes and it is more crowded in specific areas than even that suggests". Caveolae are structurally-defined, protein-rich areas of the plasma