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Multiple Estrogen Receptor Subtypes Influence Ingestive Behavior in Female Rodents

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Abstract

Postmenopausal women are at an increased risk of obesity and cardiovascular-related diseases. This is attributable, at least in part, to loss of the ovarian hormone estradiol, which inhibits food and fluid intake in humans and laboratory animal models. Although the hypophagic and anti-dipsogenic effects of estradiol have been well documented for decades, the precise mechanisms underlying these effects are not fully understood. An obvious step toward addressing this open question is identifying which estrogen receptor subtypes are involved and what intracellular processes are involved. This question, however, is complicated not only by the variety of estrogen receptor subtypes that exist, but also because many subtypes have multiple locations of action (i.e. in the nucleus or in the plasma membrane). This review will highlight our current understanding of the roles specific estrogen receptor subtypes play in mediating estradiol's anorexigenic and anti-dipsogenic effects along with highlighting the many open questions that remain. This review will also describe recent work being performed by our laboratory aimed at answering these open questions.

Keywords

Estradiol; food intake; water intake; saline intake

I. Background

Obesity rates have dramatically increased over recent decades and cardiovascular disease is the most common cause of death for women over the age of 65 in the United States [1, 2]. While environmental factors likely play an important role in these trends, hormonal changes in older women also contribute to obesity and cardiovascular disease. Estrogens, specifically estradiol, have an inhibitory influence on food, water, and saline intakes in a variety of species, including humans [3, 4]. After surgical or natural hormone withdrawal, food, water, and saline intakes significantly increase [5, 6]. Clinically, this is problematic because increased food intake is a risk factor for obesity, and increased water and saline intake can

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disturb the delicate fluid balance that is needed to maintain healthy blood pressure [7, 8]. Therefore, it is not surprising that this is of particular concern for postmenopausal women, who have low levels of estrogens and are at a greater risk for developing obesity and cardiovascular-related health problems [9–12]. Because food and fluid intakes are, respectively, important parts of energy and body fluid homeostasis, understanding how estradiol decreases these ingestive behaviors is a crucial step in the discovery of interventions that can reduce morbidity and improve quality of life, especially for women.

The inhibitory effect of estrogens on food, water, and saline intake has been well studied for decades. In a variety of species, including rats, mice, and humans, food intake fluctuates across the estrous cycle with a significant decrease in food intake around the peri-ovulatory time period [3, 13–16]. In rats, after ovariectomy baseline food intake rises and the cyclic change in intake is lost, revealing both a tonic and phasic inhibition in intake mediated by ovarian hormones [6]. Acute estradiol replacement restores the cyclic decrease in food intake [17]. Although progestin levels also fluctuate across the estrous cycle, treatment with progesterone alone has no effect on food intake nor does it modulate estradiol's ability to reduce intake. This suggests that estradiol is the primary ovarian hormone responsible for the observed effects [18–21]. In rats and mice, the decrease in food intake on estrus or after estradiol treatment is mediated by a selective decrease in meal size, often with a less reliable, and more variable, increase in meal number that fails to compensate for the reduction in intake [16, 22, 23]. Changes in meal size suggest that estradiol decreases food intake by increasing satiety signals [3]. Similar estrus-related changes in water and saline intake are also observed in female rats [5, 22, 24–26]. Again, intake increases after ovariectomy, and estradiol treatment, but not progesterone-treatment, decreases fluid intake [27–29]. Thus, there is reasonable evidence that estrogens are responsible for the ovarian effects on intake and that these effects generalize to several animal models.

Although estradiol clearly affects intake, its effects on food and fluid intake appear to occur independent of each other. An early study in ovariectomized (OVX) guinea pigs highlighted these independent effects of estradiol on ingestive behaviors [30]. In this series of experiments when food intake was restricted to ~30% below *ab libitum* levels estradiol treatment only decreased water intake. In turn, when water intake was restricted, estradiol treatment only decreased food intake. Stronger evidence for this separability of estradiol's effects on fluid intake from any effect on feeding comes from studies using dipsogenic treatments that have minimal or no effect on food intake. For instance, administration of angiotensin II (AngII) causes rapid and robust drinking behavior without any acute effects on food intake, and estradiol decreases the drinking that occurs after AngII [25, 26]. Furthermore, we recently reported that larger doses of estradiol are necessary to reduce water intake in comparison to doses necessary to reduce food intake. This demonstrates that estradiol's anorexigenic effect can be observed without a simultaneous decrease in fluid intake [31]. Together these data suggest that separate mechanisms are involved in reducing intake of food and fluids. It is, however, possible that the apparent separability of the fluid and food intake effects is due to different dose-response curves for the different behaviors, rather than separate mechanisms controlling each. Further research is required to rule out

this alternate explanation, and experiments addressing the issue are underway in our laboratory.

Fluid intake can be stimulated by reducing the volume of either the intracellular or extracellular space. Estradiol appears to selectively reduce fluid intake that is stimulated by extracellular dehydration [4]. This type of perturbation of fluid balance results in elevation of AngII, which leads to restorative ingestion of both water and saline [7]. Both water and saline intakes after AngII treatment are decreased on the day of estrus in intact female rats, and exogenous estradiol decreases AngII-stimulated intake in OVX rats [25, 26, 29, 32]. In addition to AngII treatment, other models of extracellular dehydration support the role of estrogens in controlling fluid intake. For instance, fluid intake stimulated by the β-adrenergic agonist isoproterenol varies across the estrous cycle, with the lowest intake observed during estrus [33], and estradiol treatment reduces the water and saline intakes normally stimulated by treatment with isoproterenol [28, 34, 35]. Furthermore, fluid intake stimulated by the diuretic furosemide, either alone or in combination with captopril (an inhibitor of the enzyme that generates AngII), is less robust on the day of estrus than when the treatments are given on diestrus. Consistent with the hypothesis that this is due to the elevated estradiol associated with estrus, estradiol treatment in OVX rats decreases intake normally stimulated by the diuretic with or without captopril [36, 37]. Contrary to estradiol's influence on extracellular dehydration, thirst stimulated by intracellular dehydration, which selectively increases water intake, is not reduced by estradiol. For example, water intake stimulated by hypertonic saline treatment does not change across the estrous cycle in intact rats nor is it influenced by estradiol-treatment in OVX rats [25, 34, 37, 38]. These results clearly demonstrate that estradiol has a strong effect on drinking, and that this effect is selective to drinking caused by extracellular dehydration.

A key step in generating a full understanding of the mechanism(s) by which estradiol decreases food and fluid intakes is to identify the estrogen receptor(s) involved in these processes. A complete understanding has been complicated by the existence of multiple estrogen receptor (ER) subtypes throughout the brain and their diverse cellular distribution. Specifically, ER subtypes are found in the cell nucleus, where they exert classical transcriptional effects, but are also found associated with the plasma membrane, where they act through signaling pathways with diverse effects. The remainder of this review will discuss the identified ER subtypes and will describe recent work aimed at identifying their roles in the controls of food and fluid intake in female rodents.

II. ER subtypes and localization

Until 1996 it was believed that only one ER existed. This protein, called ER, was thought to be exclusively found in the cell nucleus, where it is normally inhibited by heat shock proteins. When activated by estrogens, these heat shock proteins are released, allowing ER to dimerize, recruit coactivators/corepressors, and bind to estrogen response element sequences to initiate or repress gene expression [39, 40]. This relatively simple picture of estrogen effects was first complicated by the discovery of a new nuclear ER named estrogen receptor beta (ERβ); the original ER was renamed estrogen receptor alpha (ERα) [41, 42]. Although this finding added a new receptor that could respond to estrogens, ERβ was found

to act similarly to ERα, and the picture was only slightly expanded to include this second receptor and the clear ability of the receptors to form heterodimers [42]. Although both receptor subtypes are found throughout the central nervous system, the discovery of ERβ revealed key differences between ERα and ERβ expression in areas of the brains that are involved in the regulation of food and fluid intakes (Figure 1). For example, the subfornical organ (SFO), a region critical for fluid intake, expresses ERα, but not ERβ. The supraoptic nucleus (SON) and paraventricular nucleus (PVN), two areas also involved in regulating body fluid homeostasis, along with the dorsal raphe (DR), where the cell bodies of serotonin neurons involved in regulating food intake originate, express ERβ, but not ERα [43]. Other brain regions, such as the nucleus of the solitary tract (NTS), lateral hypothalamus (LH), arcuate nucleus (Arc), anteroventral third ventricle region (AV3V), and medial preoptic area (mPOA), express both receptor subtypes [43].

Our understanding of estrogen effects was further complicated by studies revealing the diverse mechanisms downstream of ERs. One such mechanism involves effects on cell physiology that are too rapid to require changes in gene expression. Although these rapid electrophysiological effects were identified almost 40 years ago [44], the study and identification of membrane-associated ERs only started to gain attention towards the end of the 1990s. These studies, to date, identified three novel membrane-associated ERs, GPER-1, ER-X and Gq-mER. The first of these became relevant to the study of estrogen effects when it was discovered that orphan receptor GPR30 binds estradiol and other putative ER-selective ligands without having any affinity for other steroid hormones [45, 46]. This orphan receptor was subsequently renamed G protein-coupled estrogen receptor 1 (GPER-1) because of its recognized actions through Gs and its classical heptahelical transmembrane structure. Similar to other Gs-coupled receptors, GPER-1 activates intracellular signaling through stimulation of adenylyl cyclase and G-protein-dependent release of membrane-tethered heparin bound epidermal growth factor [47]. Interestingly, GPER-1 is expressed in a number of nuclei involved in the controls of fluid and food intake. Specifically, GPER-1 mRNA has been detected in the LH, PVN, Arc and the NTS [48–50]. Immunohistochemistry studies have localized GPER-1 to neurons in the PVN and SON where it is often co-expressed with the hormone oxytocin [51], an important peptide in the control of fluid balance maintenance [52, 53]. While the search for the endogenous ligand for GPER-1 was occurring, another membrane-associated ER was identified and named ER-X. This receptor is embedded in caveolar-like microdomains where it can interact rapidly with kinases that activate a mitogen-activated protein kinase (MAPK) cascade. Interestingly, ER-X is expressed in the cortex in postnatal, but not adult, animals; however, it is re-expressed in the adult brain after injury by ischemic stroke [54]. Finally, Gq-mER is a membrane-associated G-protein coupled ER that has been extensively studied in hypothalamic tissue from guinea pigs and mice [55, 56]. A selective ligand, STX, has been developed for this receptor which, along with estradiol, causes activation of PKC-PKA pathways that can be blocked by the antagonist ICI [57]. Although there are obvious functional effects of Gq-mER in the hypothalamus, a complete mapping of its expression has been limited because the receptor has yet to be cloned (for a comprehensive review of these receptors please see [47, 58–60]).

The discovery of the membrane-associated ER subtypes revealed incredible diversity in the means by which estrogens can affect cell function. This diversity became even more robust with more recent data demonstrating that both ERα and ERβ also can associate with the cell membrane and act as surface receptors [61, 62]. This membrane association occurs through the posttranslational palmitoylation of the ER and requires interactions with caveolin proteins that anchor the receptors to the cell membrane [63, 64]. Although surface ERα/ERβ can have rapid, non-genomic effects, membrane-associated ERα and membrane-associated ERβ can still influence gene transcription via CREB phosphorylation [65–67]. This mechanism of action, however, requires additional protein interactions. Specifically, there is a growing list of studies demonstrating that membrane-associated ERα and ERβ have no intrinsic ability to activate second messenger systems but use crosstalk with other membrane receptors to provide a means for intracellular signaling. Current evidence suggests that this is often accomplished by interactions with mGluR subtypes [65–69]. For example, in hippocampal neurons, membrane-associated ERα activates mGluR5 and subsequently increases CREB activation, but membrane-associated ERα can also decrease CREB activation by interacting with mGluR3 [66]. In the Arc, membrane-associated ERα stimulates mGluR1a-mediated μ-opioid receptor internalization in the mPOA, and this internalization is critical for the expression of lordosis [67]. Additionally, mGluR1a antagonism in the Arc decreases the effect of E2 on lordosis in OVX rats [67]. Interactions between membrane-associated ERα/ERβ and mGluRs also have been shown in striatal neurons, hypothalamic astrocytes, and dorsal root ganglia neurons where they influence CREB activation or calcium influx [65, 68, 69]. These experiments have provided a crucial understanding of how ERα and ERβ have functional roles within the cell membrane in spite of an apparent lack of any direct association with intracellular signaling pathways. The diversity of ER actions is illustrated in Figure 2.

Based on the distributed expression of ER subtypes it should not be surprising that multiple nuclei involved in controlling food and water intake are areas sensitive to estradiol's anorexigenic and anti-dipsogenic effects. In the hindbrain, implants of estradiol benzoate over the caudal NTS reduce food intake in OVX rats [70]. In the midbrain, acute infusion of estradiol in the dorsal raphe nucleus reduce overnight food intake in OVX rats [71]. In the forebrain, there are multiple nuclei that have been implicated in mediating these actions of estradiol. Specifically, estradiol infusions into the Arc and mPOA reduce food intake [71, 72], and estradiol infusions into the AV3V and mPOA reduce AngII-stimulated water intake in OVX rats [38, 73]. Importantly, these responses seem to be anatomically selective because estradiol infusion into the ventromedial hypothalamus (VMH) of OVX rats does not change food or water intake [71, 73, 74], even though the VMH contains dense ER expression [43]. Figure 1 shows several structures that are involved in ingestive behaviors and highlights the ER subtype(s) expressed in each structure.

III. Food Intake: The role of ER subtypes

The majority of pharmacological and transgenic studies demonstrate that ERα is both sufficient and necessary for the anorexigenic effect of estradiol in female rats and mice. In OVX rats, acute or chronic treatment with the selective ERα agonist PPT reduces food intake by selectively reducing meal size [75, 76]. A similar reduction in food intake is

observed in OVX mice after PPT treatment [77]. Furthermore, selectively antagonizing ERα prevents exogenous estradiol's anorexigenic effect in OVX rats and the estrus-related decrease in food intake in intact female rats [78, 79]. Additional support for the role of ERα in mediating the anorexigenic effect of estradiol comes from studies in ERα knockout (αERKO) mice. αERKO mice have increased body weight and body adiposity which suggests a role for the receptor in mediating changes in food intake [80, 81]. Although baseline food intake does not differ in αERKO and wild-type mice, when αERKO mice are challenged with estradiol, a role for ERα in mediating an anorexegenic effect is revealed. Either chronic treatment with estradiol benzoate or acute treatment with PPT does not reduce food intake in OVX αERKO mice, even though these treatments are effective in wild-type mice [77, 82]. Furthermore, selective deletion of ERα from POMC neurons in the hypothalamus of wild-type female mice results in hyperphagia, demonstrating important anatomic localization for this effect [83]. Together these studies demonstrate that ERα is both necessary and sufficient for mediating estradiol's anorexigenic effect in rats and mice.

As previously discussed, ERα is traditionally described as a nuclear receptor, but it can also localize to the plasma membrane. This opens the question of whether nuclear ERα, membrane ERα, or both are involved in mediating estradiol's anorexigenic effect. We recently began addressing this issue using the OVX rat model. We measured food intake after central treatment with an estradiol-BSA conjugate; the BSA prevents the estradiol from entering the cell therefore it can only activate membrane-associated receptors. We found that overnight food intake was decreased after treatment with the estradiol-BSA conjugate [31]. This finding supports the hypothesis that membrane-associated ERs, presumably ERα, are involved in mediating the anorexigenic effect of estradiol. Ongoing research in our lab is aimed at identifying key brain areas involved in these effects and understanding how membrane receptor signaling influences food intake. Specifically, we are currently targeting the mPOA because, as discussed above, it is one area of the brain that is sensitive to both estradiol's anorexigenic and anti-dipsogenic effects. Furthermore, in an attempt to identify the relevant signaling pathways involved, we are testing for interactions between membrane-associated ERs and metabotropic glutamate receptors that may contribute to the anorexigenic effect of estradiol.

Although our studies in rats suggest that membrane-associated receptors play a role in ingestive effects of estrogens, recent transgenic mouse models suggest that although some aspects of energy homeostasis are mediated by membrane-associated ERs, the anorexigenic effects of estradiol are mediated only by nuclear receptors. Specifically, female mice that express a form of ERα that is unable to bind DNA (KIKO), thereby disrupting classical nuclear ERα signaling while leaving membrane ERα signaling intact, do not show the typical increase in body adiposity observed in traditional αERKO mice [84]. This finding suggests that membrane-associated ERα signaling is involved in energy homeostasis. On the other hand, no differences are found between KIKO and traditional αERKO mice in daily chow or high fat diet intake [84]. The lack of difference in intake is not surprising because daily chow intake in αERKO mice does not differ from wild-type mice [80, 82]. What is, perhaps, most surprising is that when the KIKO mice are OVX and challenged with estradiol, they show no change in food intake, suggesting that membrane-associated ERα signaling is not sufficient in mediating the anorexigenic effect of estradiol [85]. Reconciling

these reports from transgenic mice with our data in rats will be important in understanding the role of membrane-associated ER signaling in the control of food intake. It is possible that species-specific differences exist and membrane-associated ERα is involved in mediating changes in food intake in rats but not mice. This explanation is probable because there are already important documented differences in the estrogenic control of food intake between the two species. For example hyperphagia contributes, at least in part, to the weight gain after ovariectomy in rats [86]; however, no post-ovariectomy hyperphagia is observed in mice. In mice the body weight gain is entirely attributed to a change in energy expenditure [87]. It is, however, also possible that our estradiol-BSA study activated other membrane-associated ERs that are sufficient to decrease food intake. This, however, seems unlikely based on the pharmacological studies demonstrating that ERα is necessary for the anorexigenic effect of estradiol. Nevertheless, further study is needed to reconcile the apparently discrepant findings and determine the precise receptor involvement in the observed effects in rats.

A role for the more recently identified membrane ERs, Gq-mER and GPER-1, in the control of food intake has gained support in recent years. Chronic activation of Gq-mER with the selective agonist STX decreases average daily food intake in OVX guinea pigs and more acute treatment with STX decreases 24 h food intake to a degree similar in magnitude to estradiol [88]. Interestingly, in OVX guinea pigs, both estradiol and STX significantly reduce meal number with no change in meal size [88], demonstrating an important species difference because, as mentioned above, in rats and mice this decrease is mediated by a change in meal size. Follow up studies have shown that the inhibitory effect of Gq-mER activation on food intake is not sex dependent, but occurs in male guinea pigs as well [89]. To our knowledge, however, no studies have examined the anorexgenic effect of Gq-mER in female rats. Therefore, it will be interesting to explore whether this receptor is sufficient to decrease food intake in other rodents or if this effect is limited to guinea pigs.

Although limited, a small number of reports describe studies using knockout mice to explore the role that membrane-associated ER GPER-1 has on food intake. One study showed no change in average daily food intake in GPER-1 KO mice compared to wildtype controls, but did find a disruption in the estrus-related change in food intake [90]. In addition, the anorexigenic effect of leptin and CCK were absent in GPER-1 KO female mice, but not male mice [90]. This data suggest a role of GPER-1 in mediating E2's anorexigenic effects in female mice. Again, we are not aware of any published reports examining the role of GPER-1 in food intake in female rats, but experiments on this topic using a pharmacological approach are underway in our laboratory. Preliminary results from these studies suggest only a minimal role of GPER-1 in controlling food intake in OVX rats, providing additional support to the hypothesis that ERα is the most critical receptor for the anorexigenic effect of estradiol in female rats.

There is little available support for a role of either ER-X or ERβ in contributing to a change in food intake in rodents. To our knowledge there are no reports which have examined the contribution of ER-X to the anorexigenic effect of estradiol. The expression pattern alone of ER-X, which is detected either early in development or after brain injury, rules out any significant involvement it might have in regulating food intake in healthy adults [54]. There

have been, however, a number of studies examining the role of ERβ in the estrogenic control of food intake. Pharmacological studies in female rats show that acute or chronic activation of ERβ is not sufficient to decrease food intake [75, 76] and selective antagonism of ERβ does not prevent estradiol's inhibitory effect on food intake [79]. There are, however, two reports that suggest some support for a role of ERβ in the controls of food intake in females. In one report, antisense oligodeoxynuleotides directed against ERβ, but not ERα, prevented a decrease in food intake following estradiol treatment in OVX rats [91]. A second study reported no change in food intake after estradiol treatment in OVX βERKO mice, suggesting the receptor is necessary for this effect [92]. It is unclear how to reconcile the data from these two studies with the numerous pharmacological and other knockout reports suggesting a minimal role of ERβ in mediating the anorexigenic effect of estradiol. Accordingly, the bulk of the evidence continues to point to the ERα subtype as the most critical for the regulation of feeding behavior.

IV. Water and Saline Intake: The Role of ER Subtypes

In contrast to the plethora of research focused on understanding the role of ER subtypes in mediating changes in food intake, almost no research has focused on understanding which ER subtypes mediate changes in water and saline intake. To our knowledge, fluid intake measures have not been reported using any ER knockout mouse model, and fluid intake control by estrogens appears to be almost exclusively studied in the female rat. One recent study tested the hypothesis that estradiol can rapidly reduce water intake. Although this is not a direct test of which receptor subtypes are involved, understanding the timecourse of action can shed light into the properties of the receptors that contribute to estradiol's anti-dipsogenic effect. In this study, the authors reported no difference in short term (15 min–90 min) water intake stimulated by isoproterenol in oil- and estradiol-treated OVX rats. The earliest observable effects of estradiol were not apparent until 24 h after treatment [93]. This suggests that transcriptional events are required for the decrease in fluid intake. Because nuclear receptors can influence gene expression directly, and membrane-associated receptors can influence gene expression indirectly, it is possible that any ER could be involved in mediating this change. Studies described above conducted by our laboratory using an estradiol-BSA compound provide some relevant information. The reduction of overnight water intake after treatment with the estradiol-BSA provides support for a role of membrane-associated ERs [31]. Furthermore, we performed licking microstructure analysis after rats were treated with estradiol or estradiol-BSA that provides an important test for differences between the effect of the membrane-only effect of the BSA compound and estradiol that will activate all ERs. These analyses revealed that the anti-dipsogenic effect of estradiol was mediated by a change in both burst number and burst size, suggesting respective changes in post-ingestive and orosensory aspects of water intake, but the decrease in water intake after estradiol-BSA treatment was mediated only by a change in post-ingestive signals. Together this suggests that both nuclear and membrane-associated ERs are involved in reducing fluid intake, but perhaps through different mechanisms. Ongoing research in our laboratory is aimed at understanding which ER subtypes are involved in the anti-dipsogenic and anti-natriorexigenic effects of estradiol. Specifically, we are using a variety of approaches to investigate the roles of ERα, ERβ and GPER-1 in the control of

water and saline intake in female rats. Our preliminary data support different roles for each of the three receptors in the control of fluid balance.

V. Conclusions

Estrogens have obvious effects on behavior and health, and regulation of food and fluid intakes is particularly critical for energy homeostasis and cardiovascular health. Therefore, understanding the role estrogens play in food and fluid intake could lead to better therapies to reduce morbidity and improve quality of life for postmenopausal women. This review has focused on previous studies examining the role of ERs in mediating estradiol's anorexigenic, anti-dipsogenic and anti-natriorexigenic effects. Although we have described a number of important studies, and noted some progress toward an understanding of the ways that estrogens affect intake, we believe that more than anything, our discussion shows a disappointing sparsity of studies on these questions, and highlights the many open questions that remain. Ongoing research in our laboratory is designed to address many of these open questions. Furthermore, a recent strategic plan by the National Institutes of Health which aims to increase research on women's health issues has set forth a number of goals, including: (1) increasing the study of sex and gender differences in basic biomedical and behavioral research, (2) incorporating findings of sex and gender differences into the design and application of new technologies, medical devices, and therapeutic drugs, and (3) employ innovative strategies to build a well-trained, diverse, and vigorous women's health research workforce [94]. We are cautiously optimistic that this plan will help attract more investigators to this area of research. In addition to this more direct translational relevance, studies of estrogens on food and fluid intake serve as an important basic model for understanding broader questions related to behavioral effects of steroid hormones.

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Highlights

- This review focuses on estrogen effects on ingestive behaviors
- Estrogens affect intake through several receptor subtypes
- The relevant estrogen receptors are found in several cellular locations
- Estrogens affect intake by actions at several brain areas
- Work from our laboratory has helped identify relevant estrogen receptor subtypes

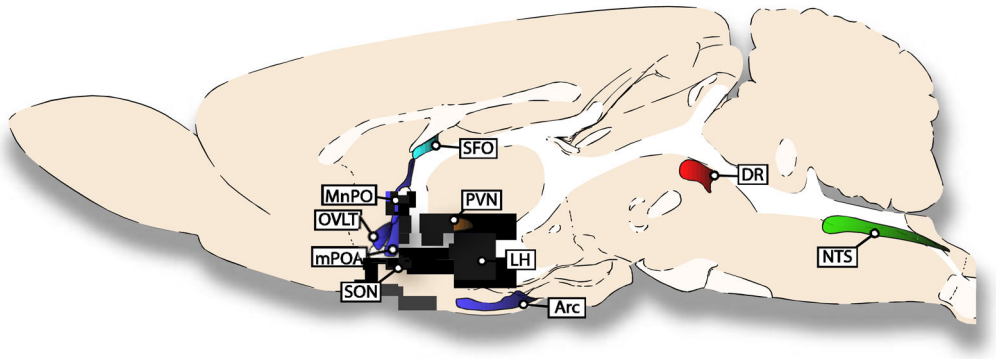

Figure 1.

Select brain structures that express ER subtypes and are involved in the effect of estrogens on ingestive behaviors. The ER subtype mRNA expressed in each area of rat brain is indicated by the color of the structure. SFO only expresses ERα (blue), whereas ERβ is the only ER subtype found in the DR (red). The MnPO, OVLT, mPOA, LH and ARC express both ERα and ERβ (purple). ERβ and GPER-1 are expressed in the PVN and SON (orange). ERα, ERβ and GPER-1 are expressed in the NTS (green).

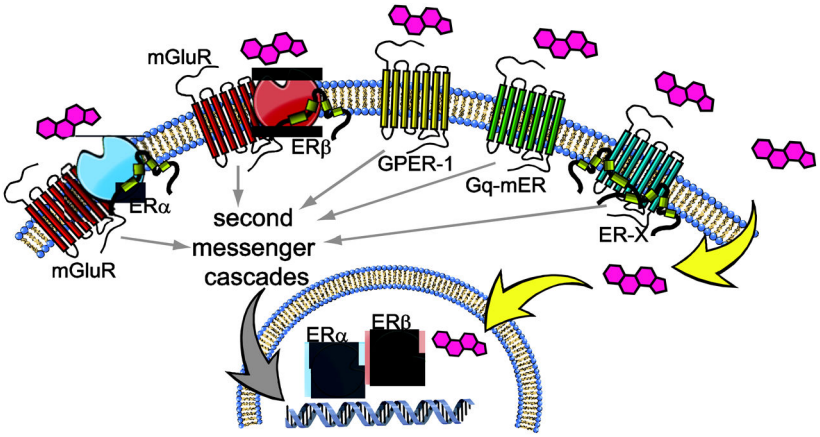

Figure 2.

Estrogens act through a diverse set of receptor subtypes. The ERα and ERβ subtypes are found in the nucleus of the cell, where they respond to estrogens to alter gene expression. After posttranslational modification, both ERα and ERβ are found associated with the plasma membrane, where they form a complex that includes caveolin and mGluR subtypes. Estrogens can also act at membrane-associated GPER-1, Gq-mER, and ER-X receptor subtypes, the latter of which is found embedded in caveolar-like microdomains. The membrane-associated receptors are able to exert rapid effects by interactions with ion channels, and slower effects through second messenger cascades that affect transcription.