Membrane estrogen receptor-alpha interacts with metabotropic glutamate receptor 1a to stimulate intracellular calcium release and progesterone synthesis in female hypothalamic astrocytes

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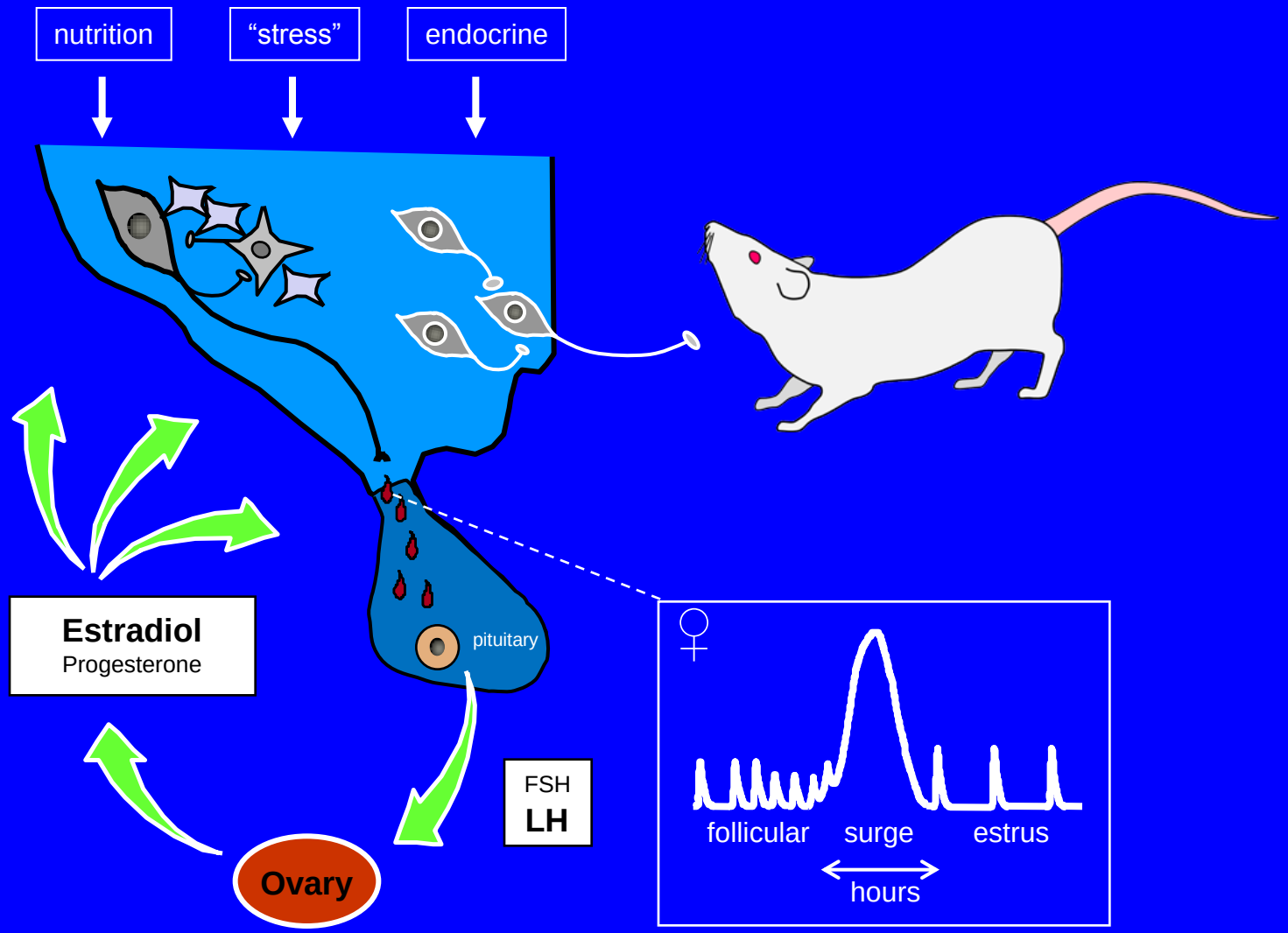
Main Outline

- Importance of hypothalamic progesterone in ovulation.
- Neuroprogesterone production by astrocytes.
- Several membrane-associated estrogen receptors and their rapid signaling pathway.
- Membrane-associated estrogen receptors interact with metabotropic glutamate receptors.
- Sex differences in hypothalamic astrocytes.

Neurobiology of Fertility Control



Role of Progesterone in Ovulation

- Circulating estradiol induces the synthesis of PRs in the rat hypothalamus. (MacLusky and Naftolin, ***Science*** 1978)
- A pre-ovulatory rise in progesterone and PR activation are obligatory for the GnRH and LH surges in rats. (Rao and Mahesh, ***Biol Reprod*** 1986; Chappell and Levine, ***Endocrinology*** 2000)

OVX rat

+

sc estradiol

= **LH Surge**

OVX rat

+

sc PR antagonist
or
3V PR antisense ODN

+

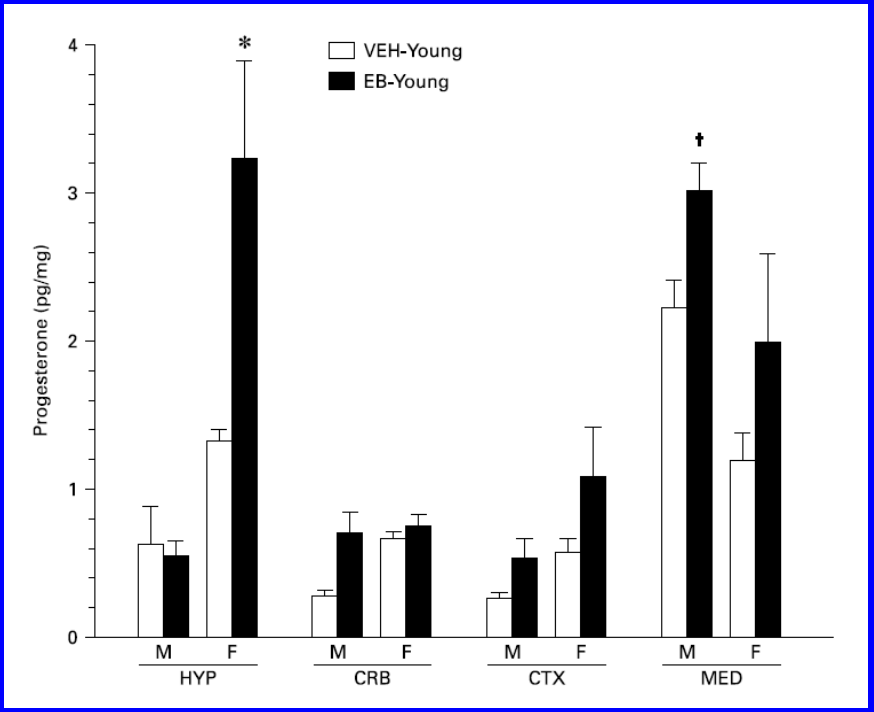
sc estradiol

= **LH Surge** ✗

Source of Neuroprogesterone

Pre-LH surge progesterone is synthesized centrally:

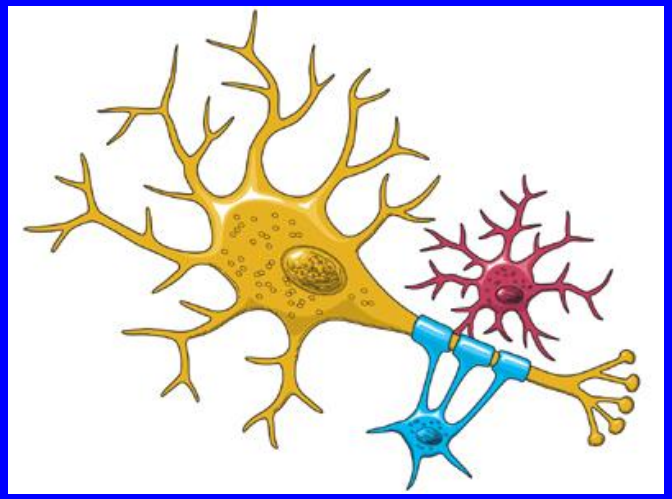
- Peripheral progesterone does not rise prior to the LH surge. (Kalra and Kalra, ***Endocrinology*** 1974)
- Exogenous estradiol increases hypothalamic neuroprogesterone in OVX/ADX rats.

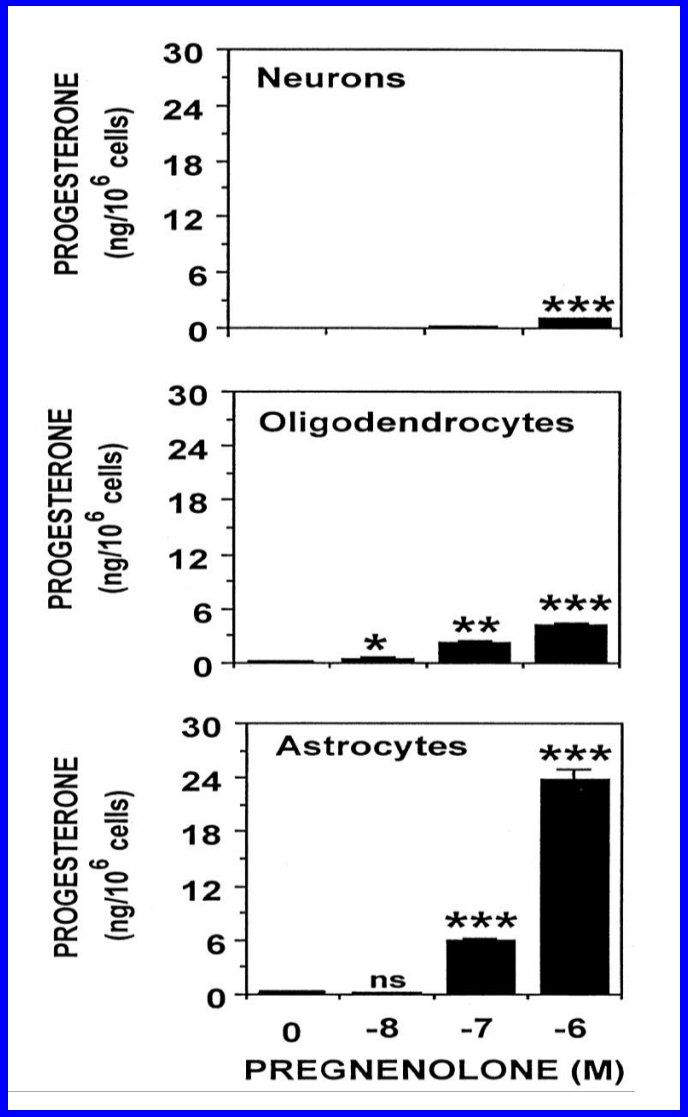


Micevych et al., ***Neuroendocrinology*** 2003

Source of hypothalamic progesterone

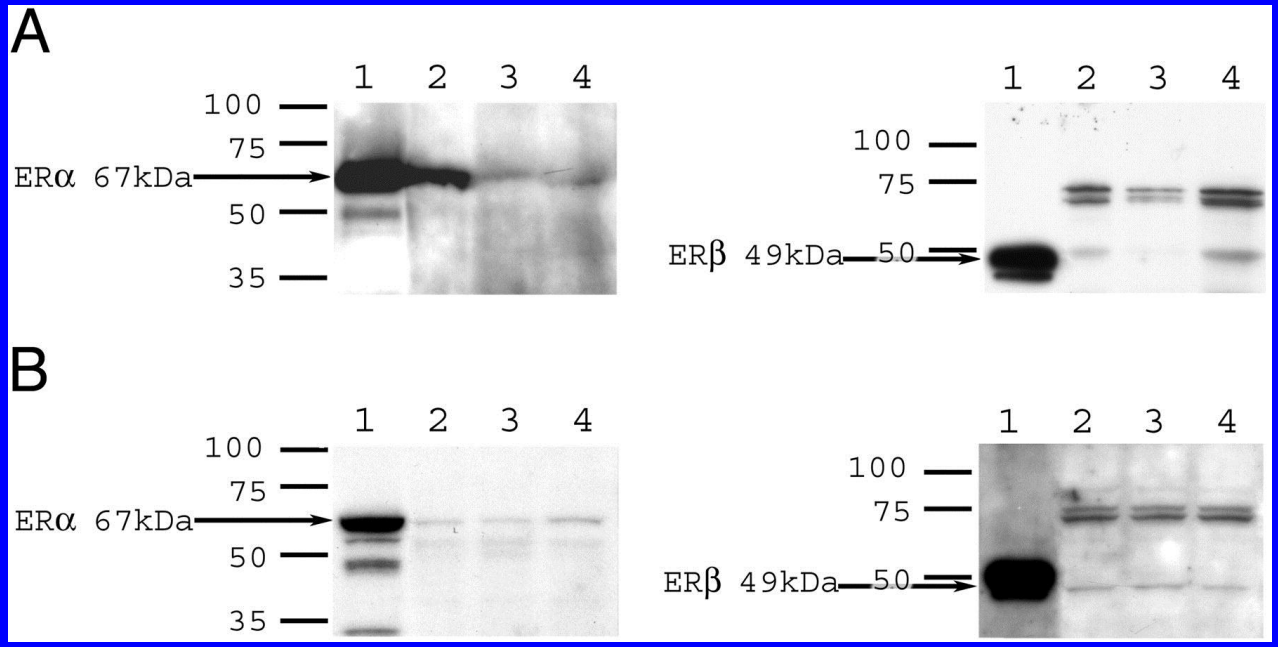
- Astrocytes are the most steroidogenically active cells in the brain.
- Astrocytes are the major producers of progesterone.





Zwain and Yen., ***Endocrinology*** 1999

Astrocytes express membrane-associated ERα and ERβ



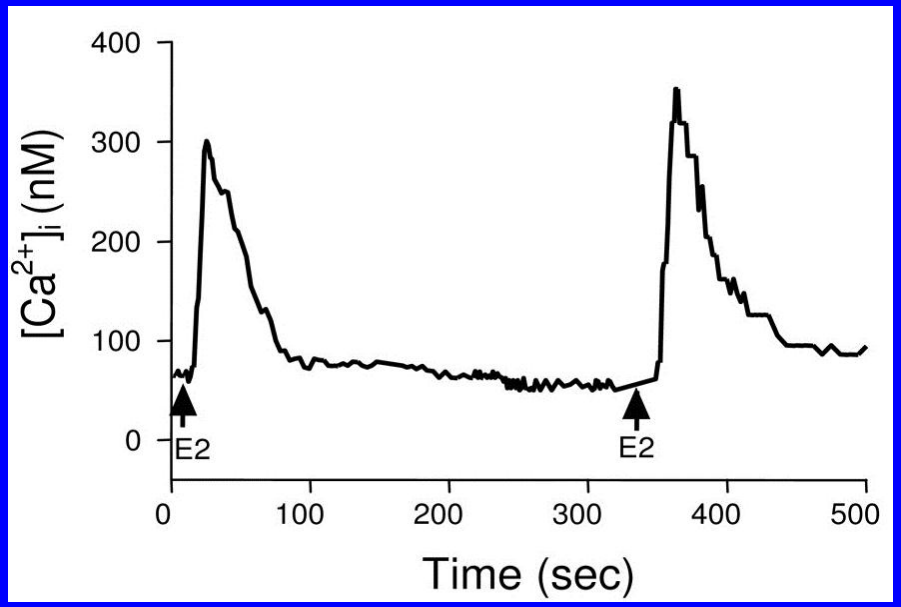
Chaban et al., ***Endocrinology*** 2004

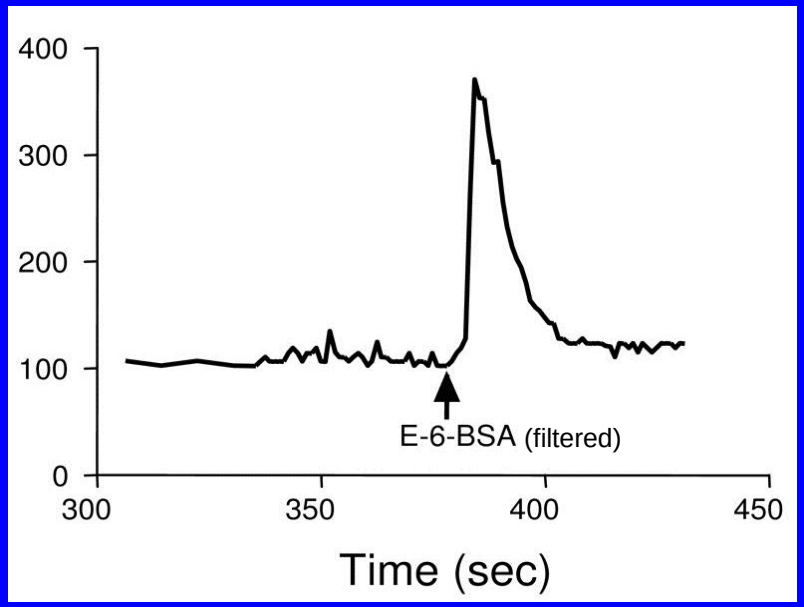
- Western Blot indicates that both ER-α and ER-β are present in the plasma membrane (A) as well as cytosolic/nuclear fractions (B) in rat neonatal cortical astrocytes.

Membrane Estrogen Receptors

E-6-BSA is a membrane-impermeable estradiol-bovine serum albumin construct.

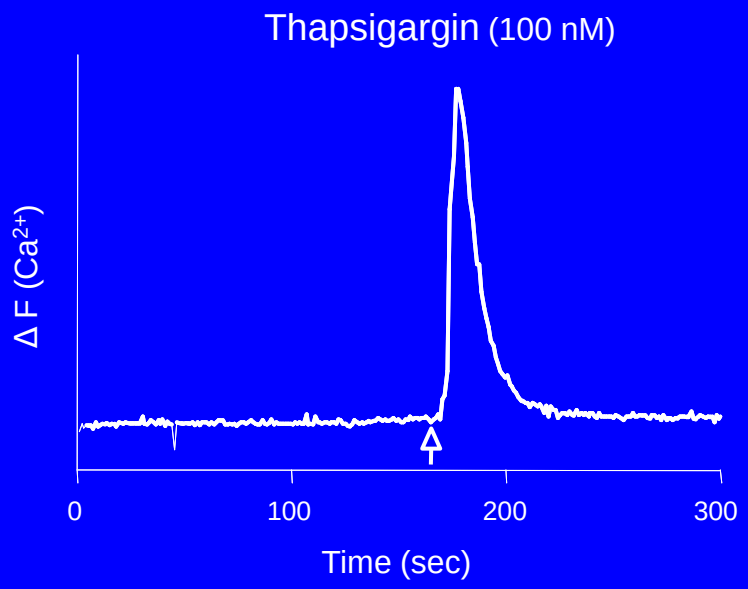
- It produces a similar amplitude of response as estradiol.
- Suggests that estradiol may act on a plasma membrane-associated ER.

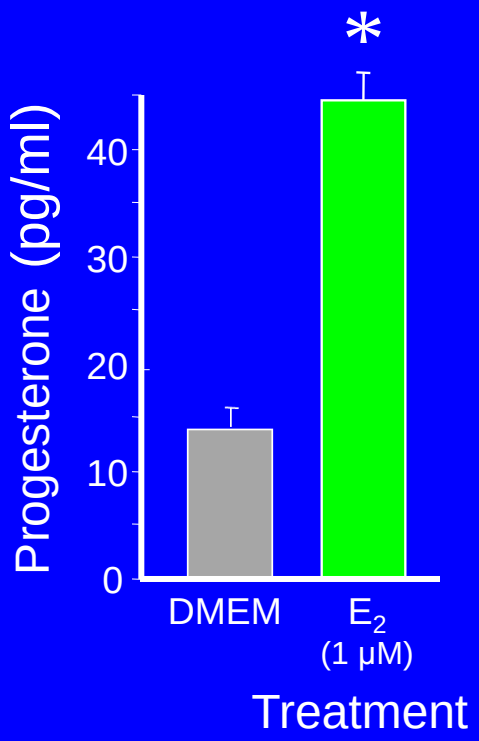




Micevych et al., ***Endocrinology*** 2007

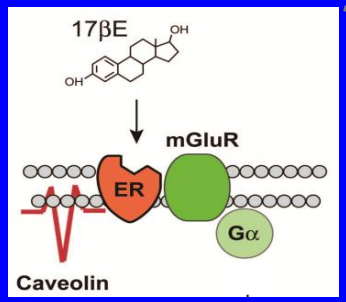
Progesterone synthesis is Ca^{2+}-dependent in post-pubertal hypothalamic astrocytes



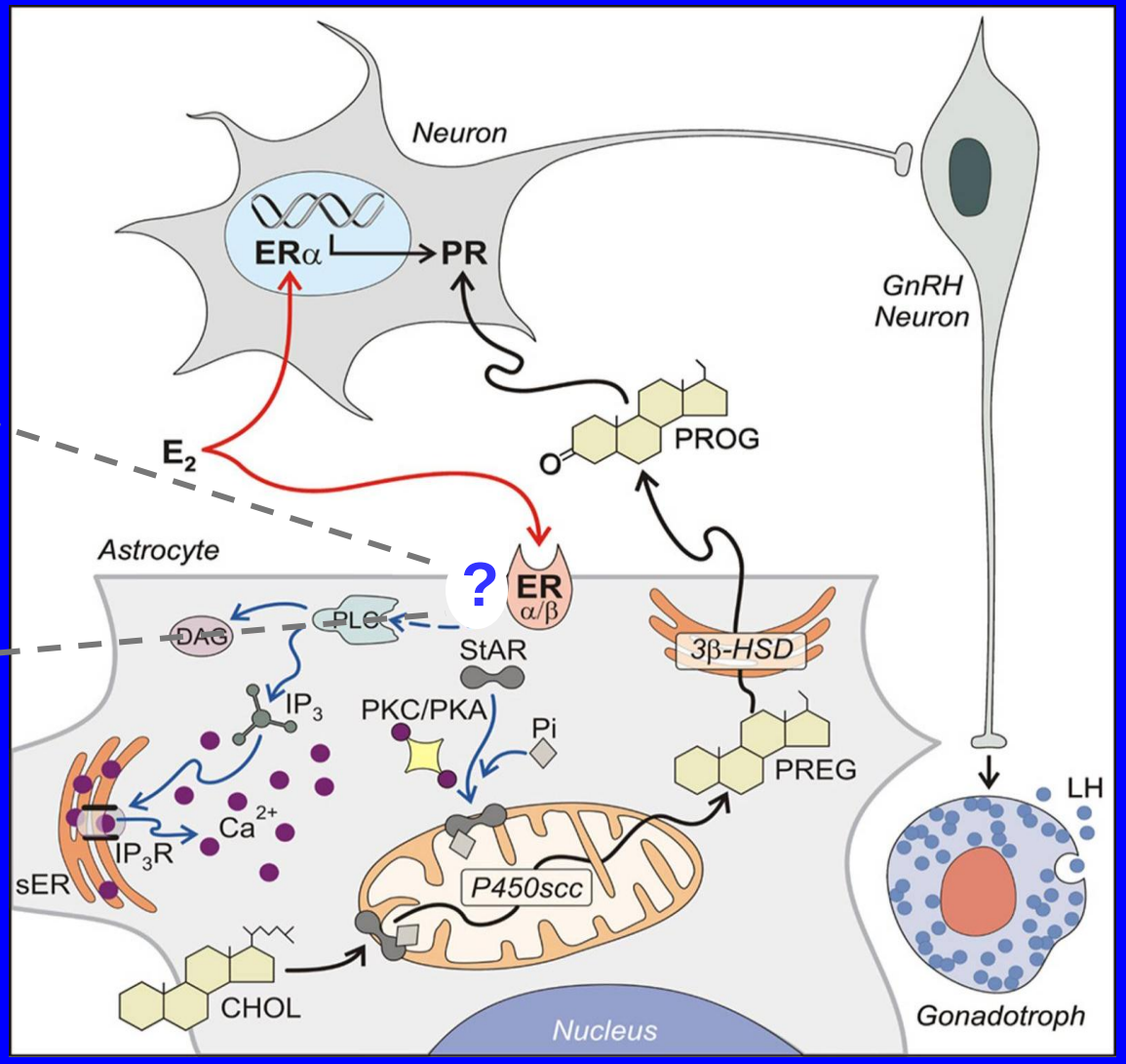


Micevych et al., ***Endocrinology*** 2007

Model of estradiol regulation of the LH surge

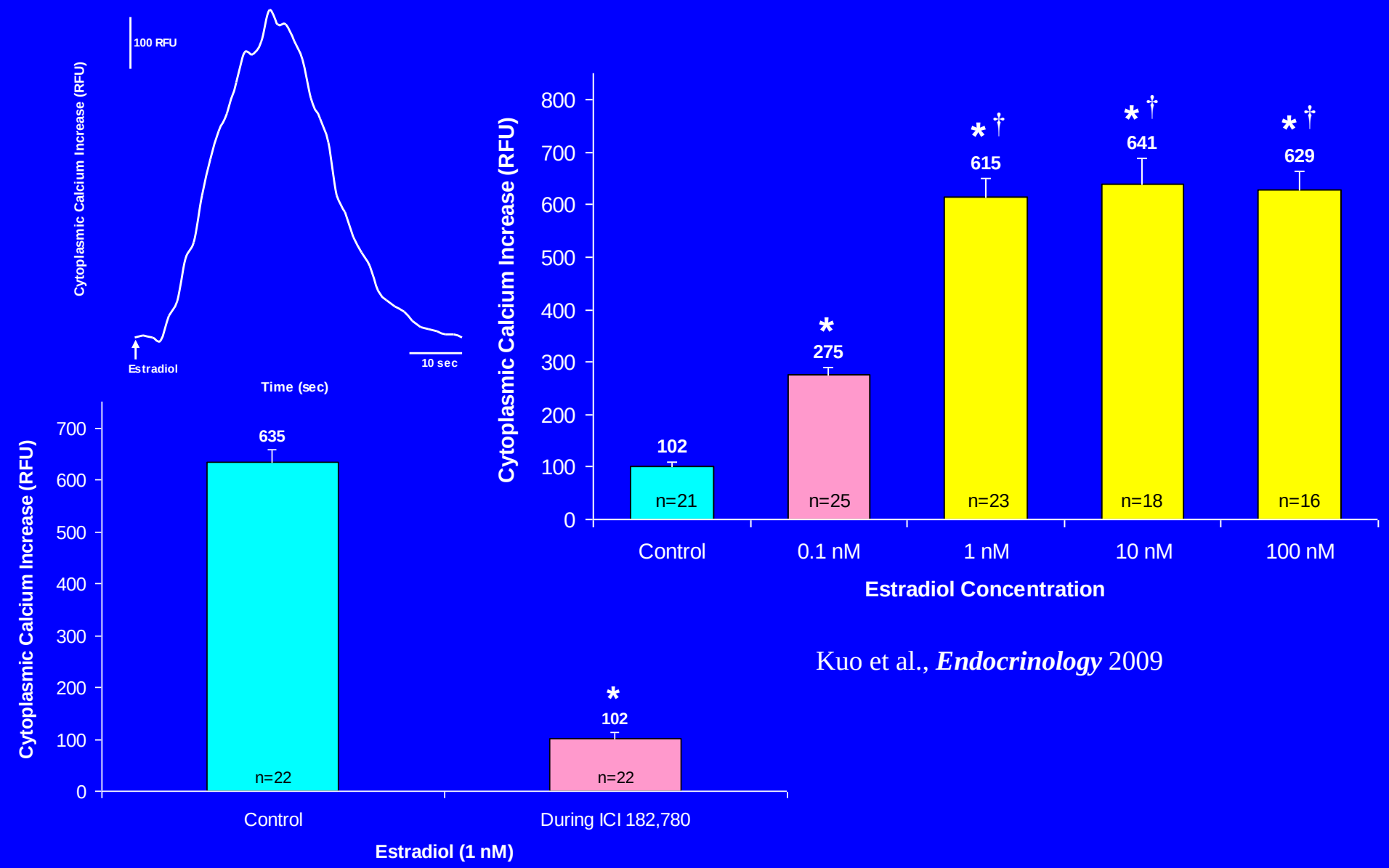


Boulware et al., ***J Neurosci*** 2007



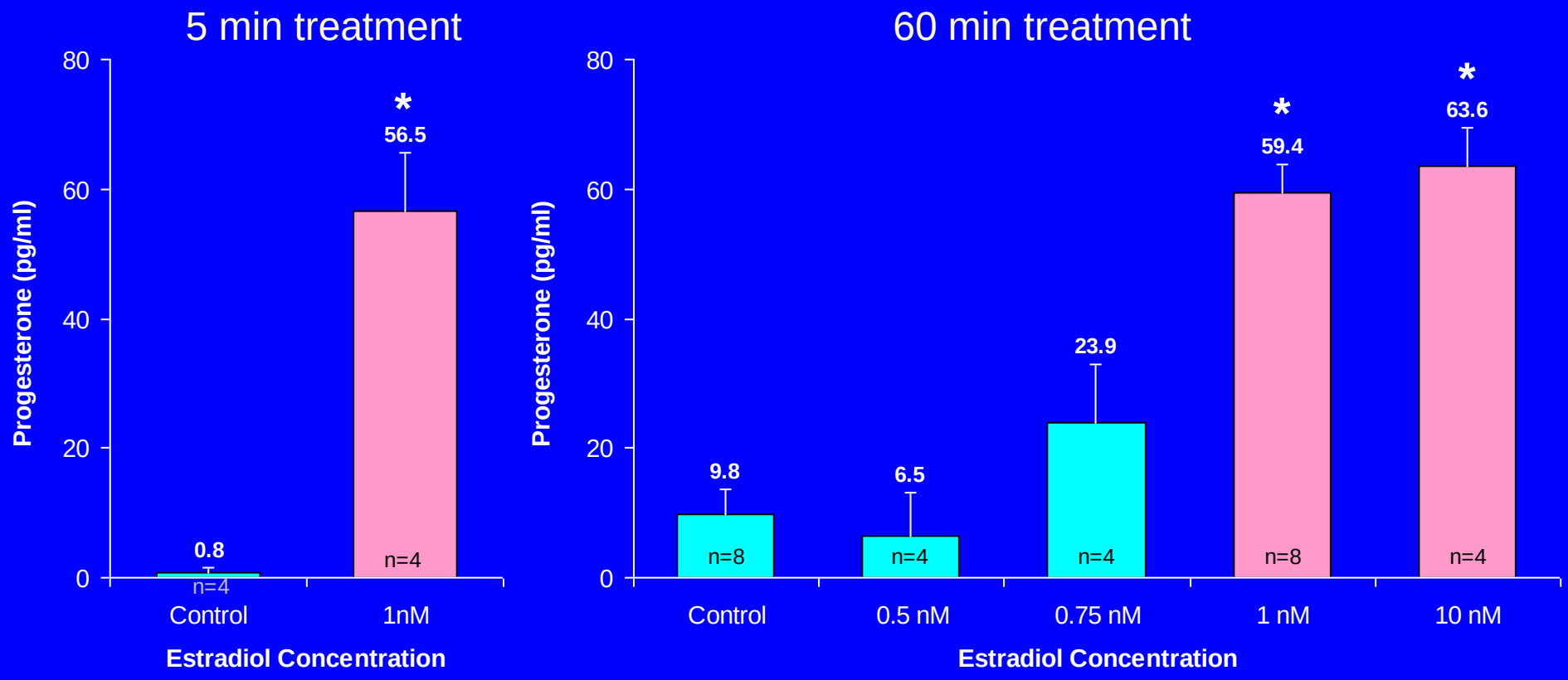
Micevych & Sinchak, ***Endocrinology*** 2008

Estradiol induces rapid elevation of free cytoplasmic calcium ($[Ca^{2+}]_i$) in hypothalamic astrocytes.



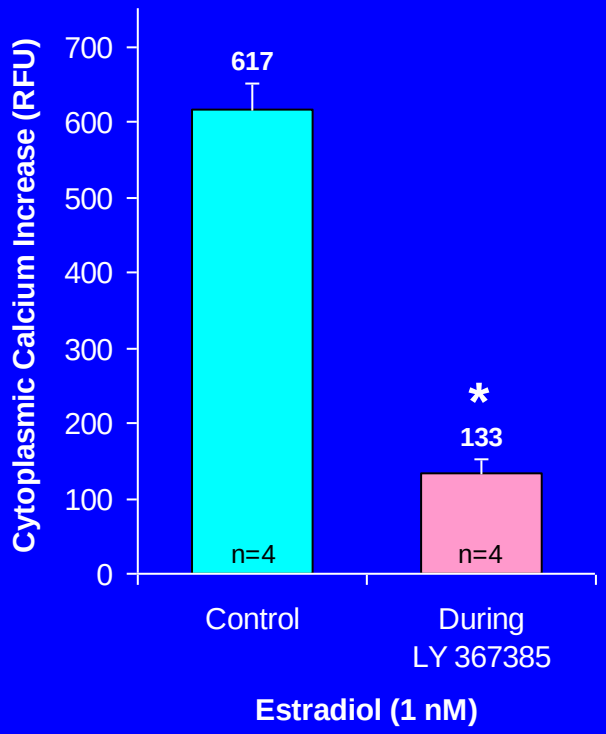
Kuo et al., ***Endocrinology*** 2009

Estradiol rapidly increases progesterone synthesis.

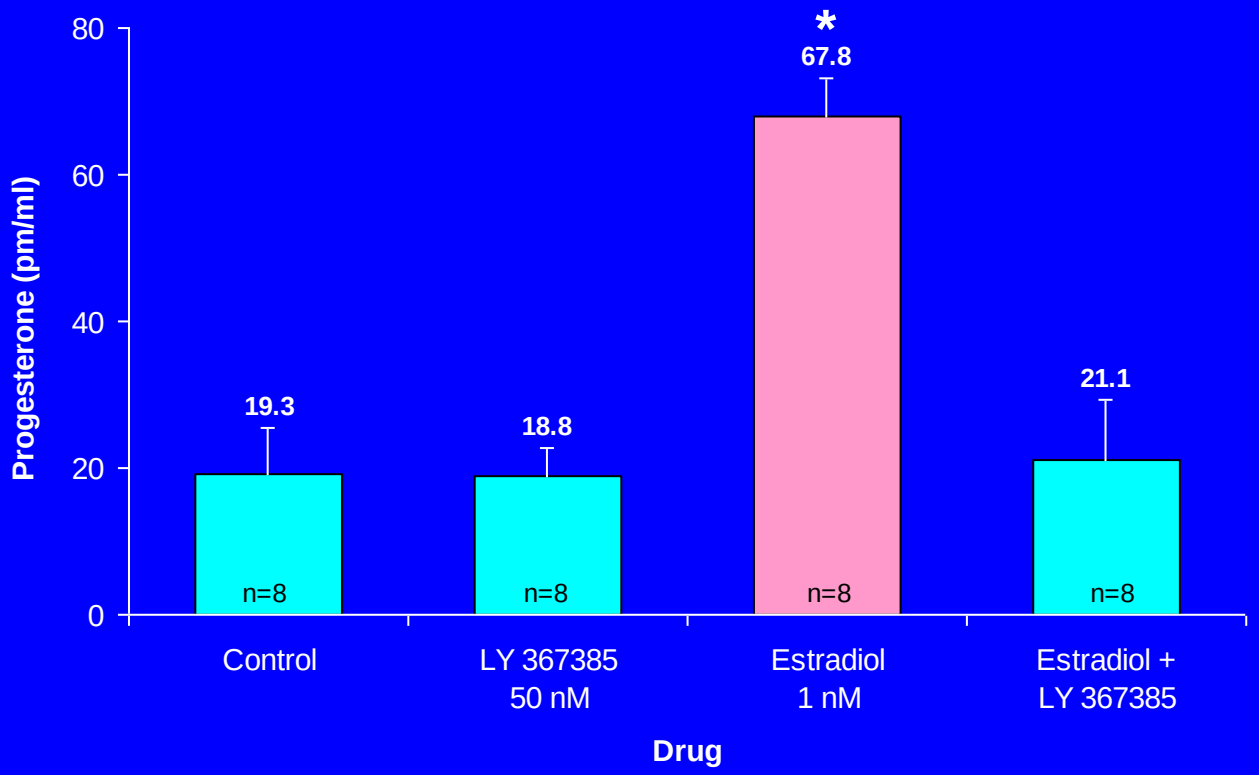


Kuo et al., ***J Neurosci*** 2010

mGluR1a antagonism attenuates the estradiol-stimulated $[Ca^{2+}]_i$ elevation & progesterone synthesis.

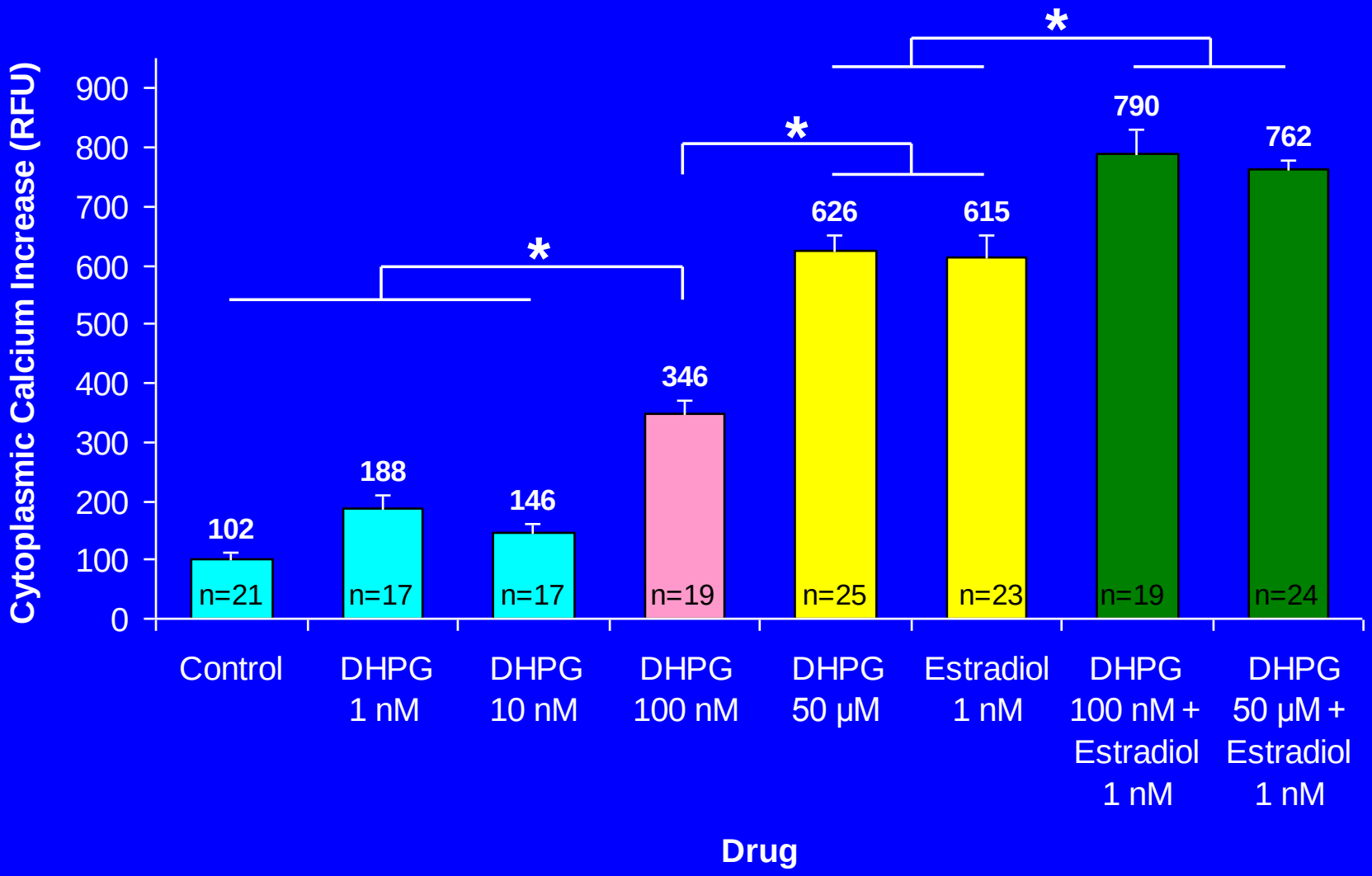


Kuo et al., ***Endocrinology*** 2009



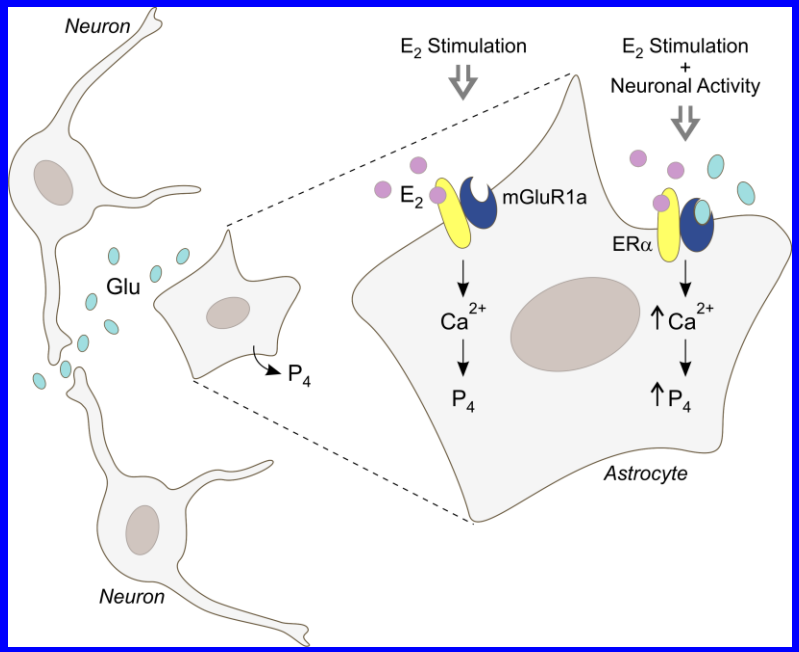
Kuo et al., ***J Neurosci*** 2010

Maximal response of $[Ca^{2+}]_i$ elevation with DHPG & estradiol are similar, but additive in combination.



Kuo et al., ***Endocrinology*** 2009

Combined estradiol & DHPG facilitate progesterone synthesis.



Kuo et al., ***J Neurosci*** 2010

Summary of Findings

- Estradiol stimulates a rapid elevation of free cytoplasmic calcium & progesterone synthesis in female hypothalamic astrocytes.
- Membrane ER signaling requires mGluR1a.

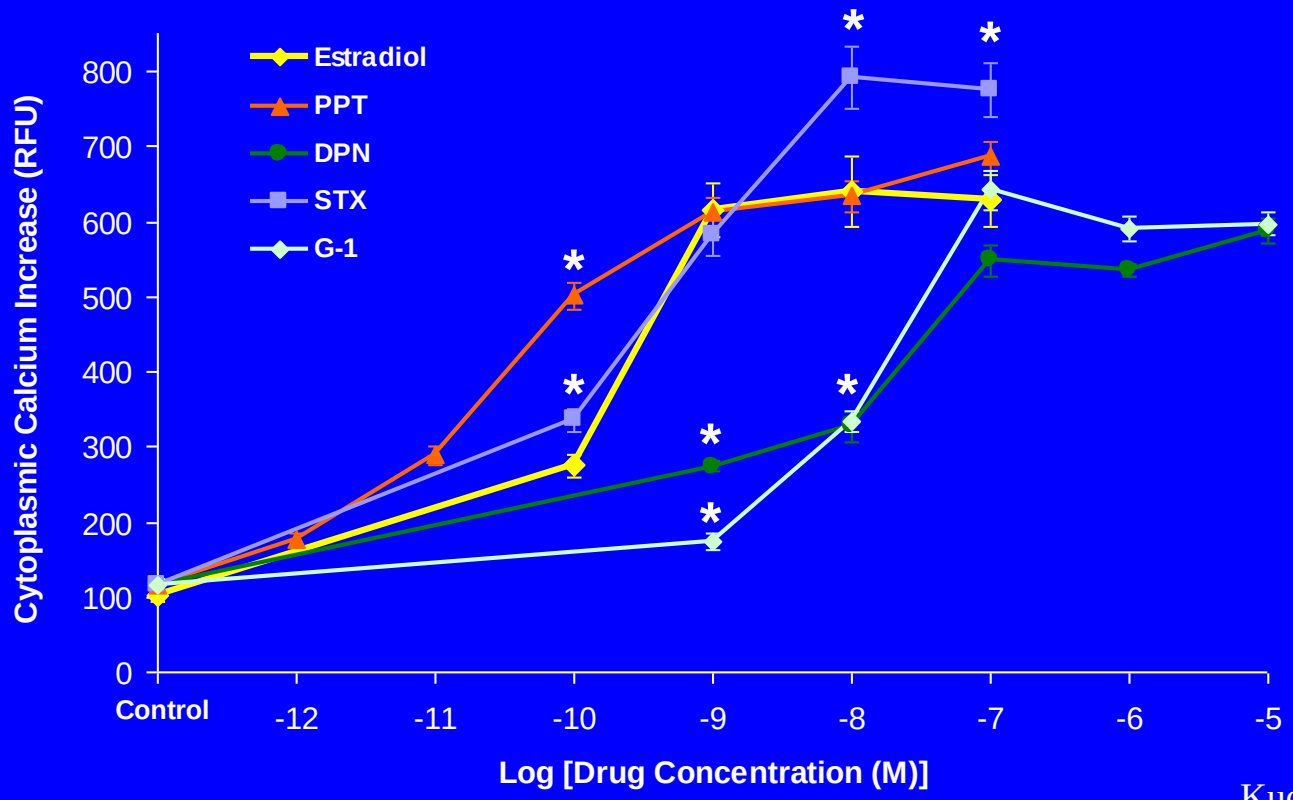
Membrane Estrogen Receptors

First evidence from 1960's, EP studies revealed membrane hyperpolarization of hypothalamic neurons within seconds of 17β-estradiol exposure.

Several candidate receptors:

- ERα and ERβ (same as nuclear receptors) signals through mGluRs. (Razandi et al, ***Mol Endocrinol*** 1999; Boulware et al, ***J Neurosci*** 2005)
- GPR30 (GPCR) activity in cells lacking ERα/ERβ, but controversial membrane location. (Filardo et al., ***Mol Endocrinol*** 2000; Revankar et al., ***Science*** 2005)
- STX-binding protein (G-protein coupled, structure unknown) with activity in ERα/ERβ double knockouts, but antagonized by ICI 182,780. (Qiu et al, ***J Neurosci*** 2003)
- ER-X (ERα sequence homology), but only reported in P7 mice and after ischemic injury, preferentially activated by 17α-estradiol, and not antagonized by ICI 182,780. (Toran-Allerand et al, ***J Neurosci*** 2002)

Comparison of estradiol with agonist for other putative mERs.



E2: EC_{50} = 0.15 nM

PPT: EC_{50} = 0.03 nM

DPN: EC_{50} = 2.55 nM

STX: EC_{50} = 0.21 nM

G-1: EC_{50} = 10.7 nM

Kuo et al., ***J Neurosci*** 2010

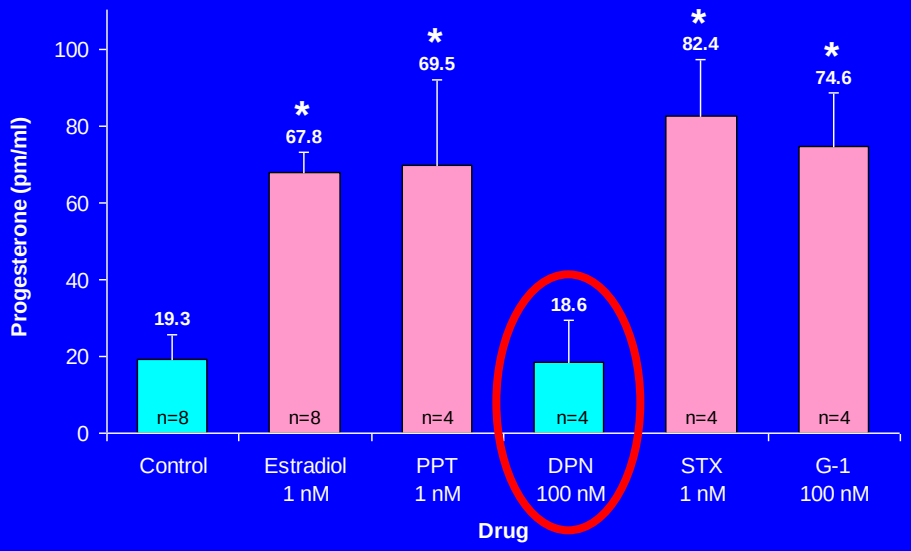
PPT = selective ERα agonist

DPN = selective ERβ agonist

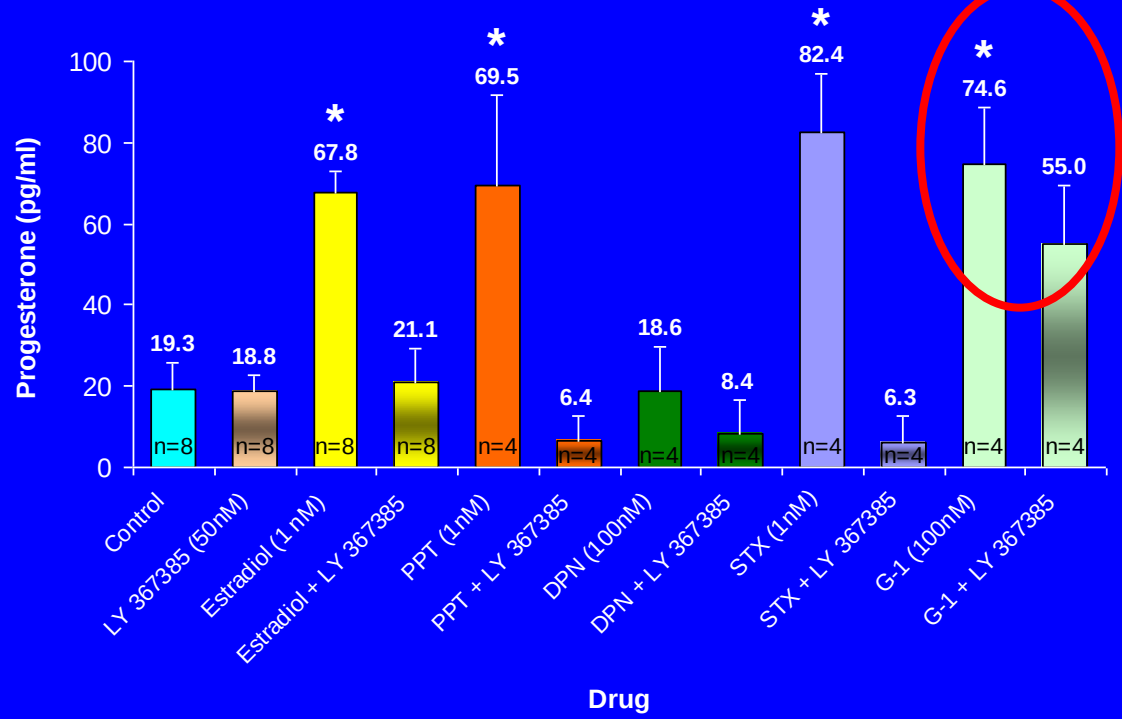
STX = STX-binding protein agonist

G-1 = GPR30 agonist

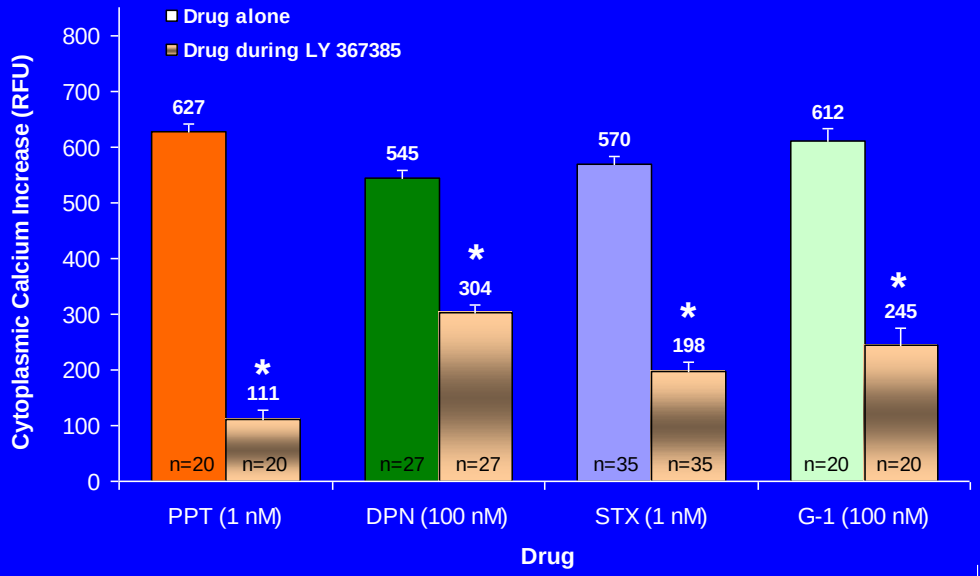
Comparison of putative mERs agonists.



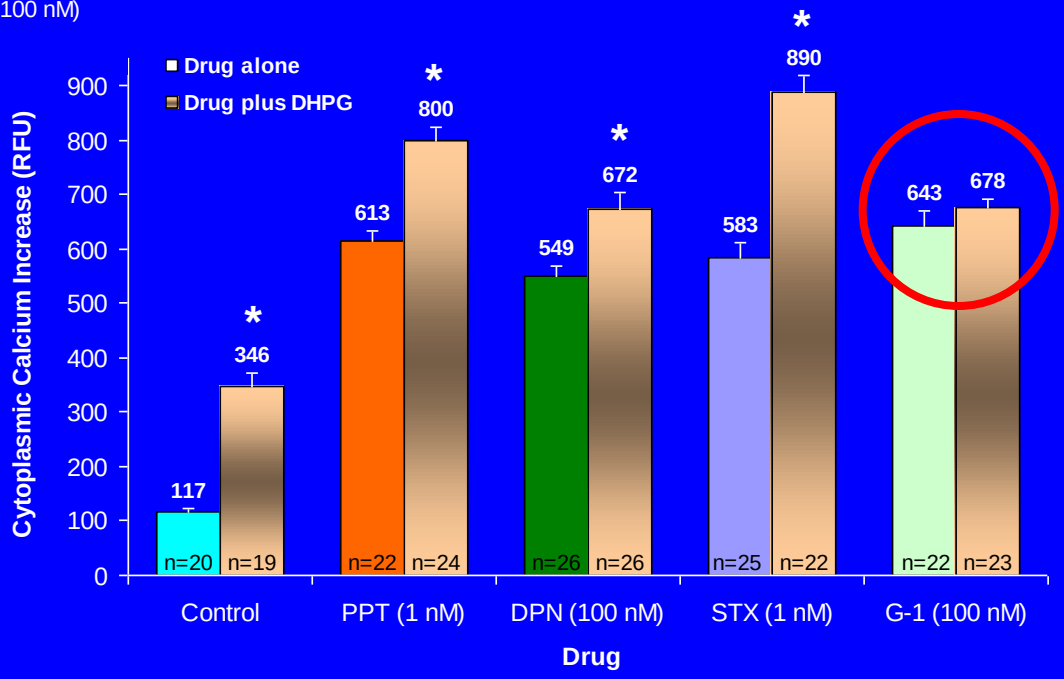
Kuo et al., ***J Neurosci*** 2010



mGluR1a antagonist and agonist on estradiol-induced-$[Ca^{2+}]_i$ release.



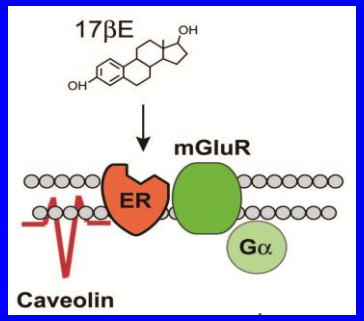
Kuo et al., ***J Neurosci*** 2010

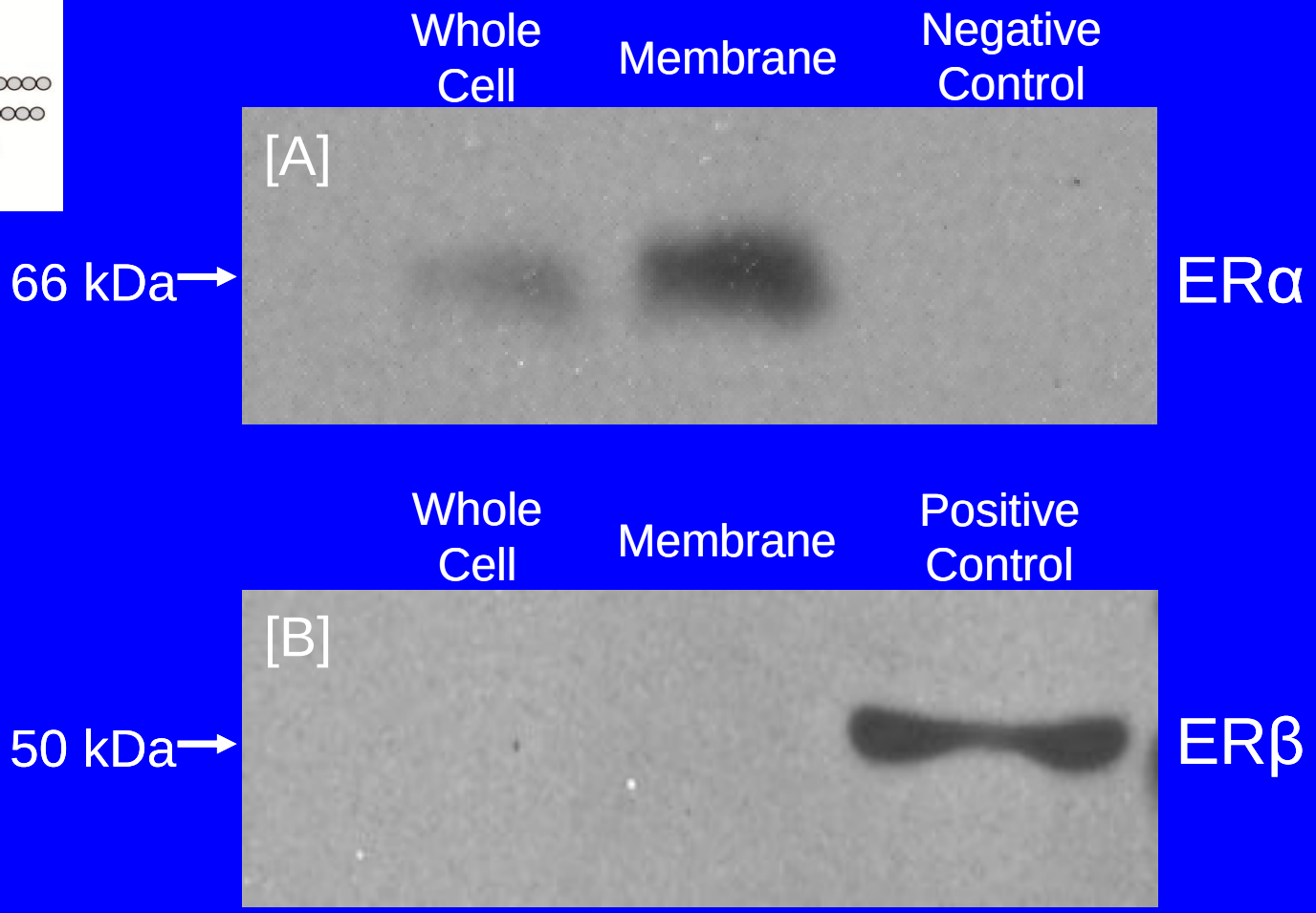


Summary of Findings

- PPT and STX most closely mimicked estradiol's effects, suggesting that estradiol may signal through mERα and/or STX-binding protein.
- DPN failed to stimulate progesterone synthesis and required high doses for $[Ca^{2+}]_i$ release. Thus, estradiol is not signaling through mERβ.
- GPR30 signals through an unique pathway.

Co-immunoprecipitation shows ERα-mGluR1a interaction, but not ERβ-mGluR1a interaction.

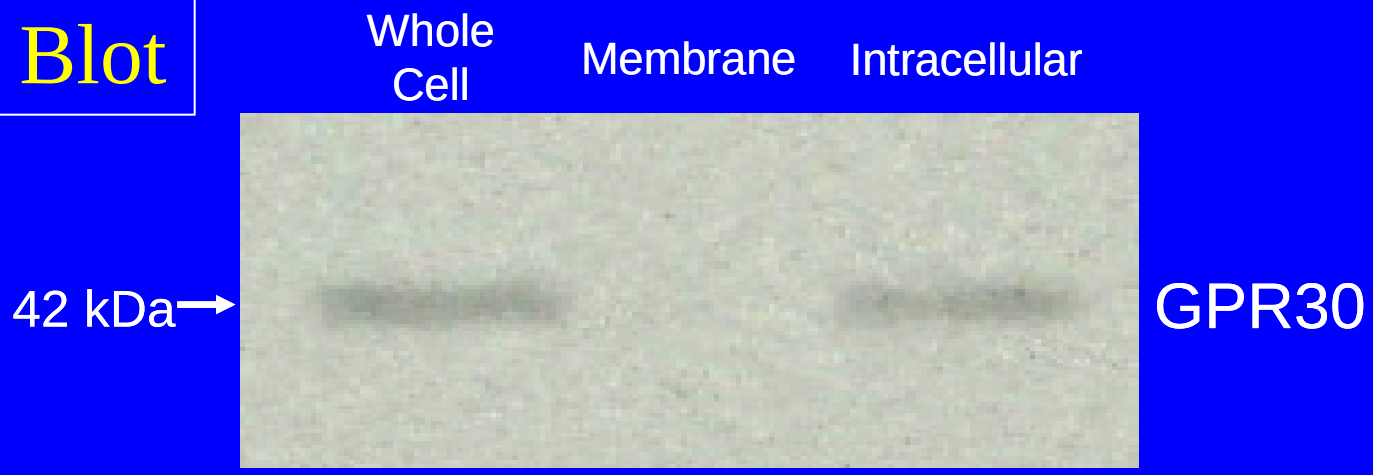




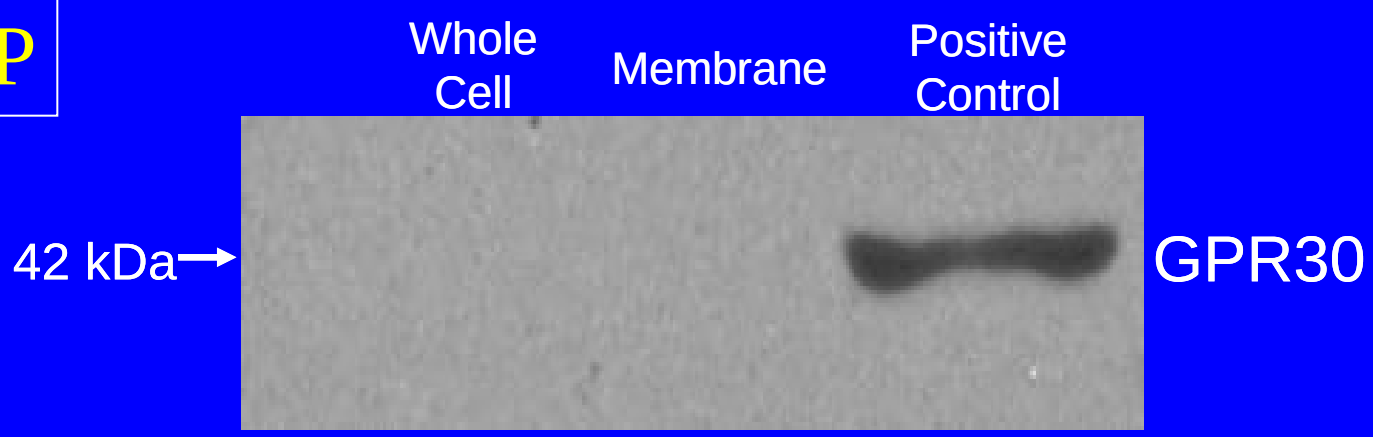
Kuo et al., ***Endocrinology*** 2009; Kuo et al., ***J Neurosci*** 2010

Results: GPR30 is expressed in astrocytes but does not co-immunoprecipitate with mGluR1a.

Western Blot



Co-IP



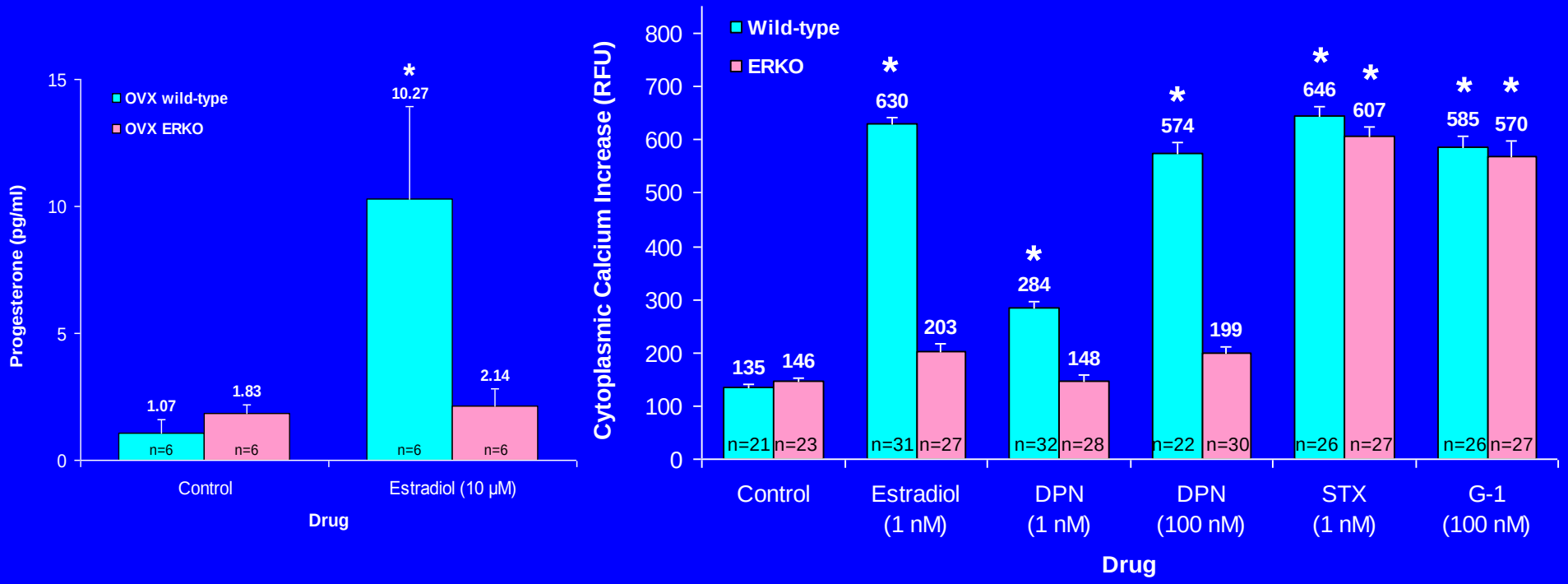
Kuo et al., ***J Neurosci*** 2010

Summary of Findings

- ERα, but not ERβ or GPR30, interacts with mGluR1a as demonstrated by co-IP.
- GPR30 was not identified on the plasma membrane.
- G-1 stimulated progesterone synthesis was not attenuated by LY 367385, suggesting that G-1 may activate an intracellular GPR30 to directly release $[Ca^{2+}]_i$ and stimulate progesterone synthesis.
- G-1 required high doses for $[Ca^{2+}]_i$ release, which was blocked by LY 367385 but not enhanced by DHPG, suggesting that G-1 may also act on the mGluR1a.

ERKO mice confirm estradiol action through ERα.

ERα knockout mice



Kuo et al., ***J Neurosci*** 2010

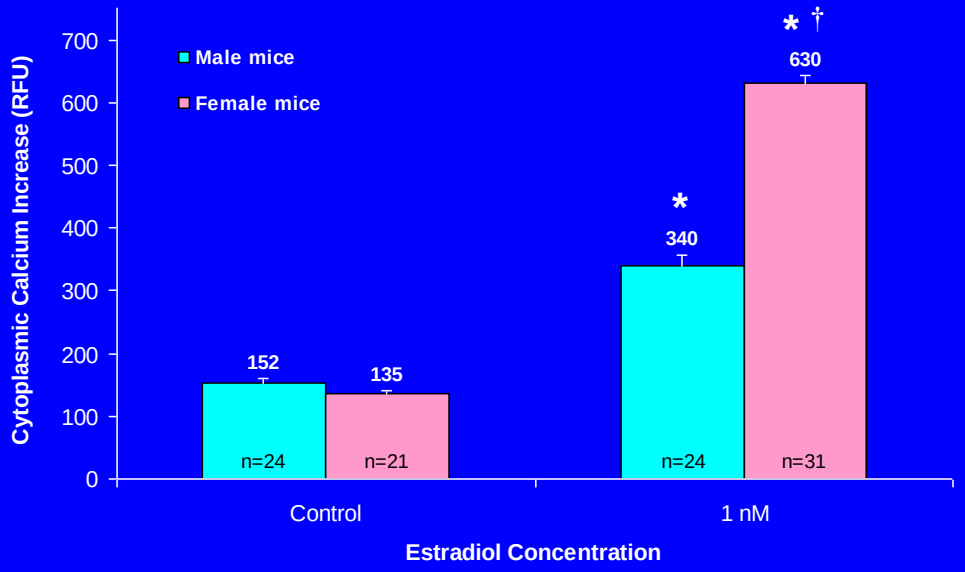
Summary of Findings

In ERKO mice:

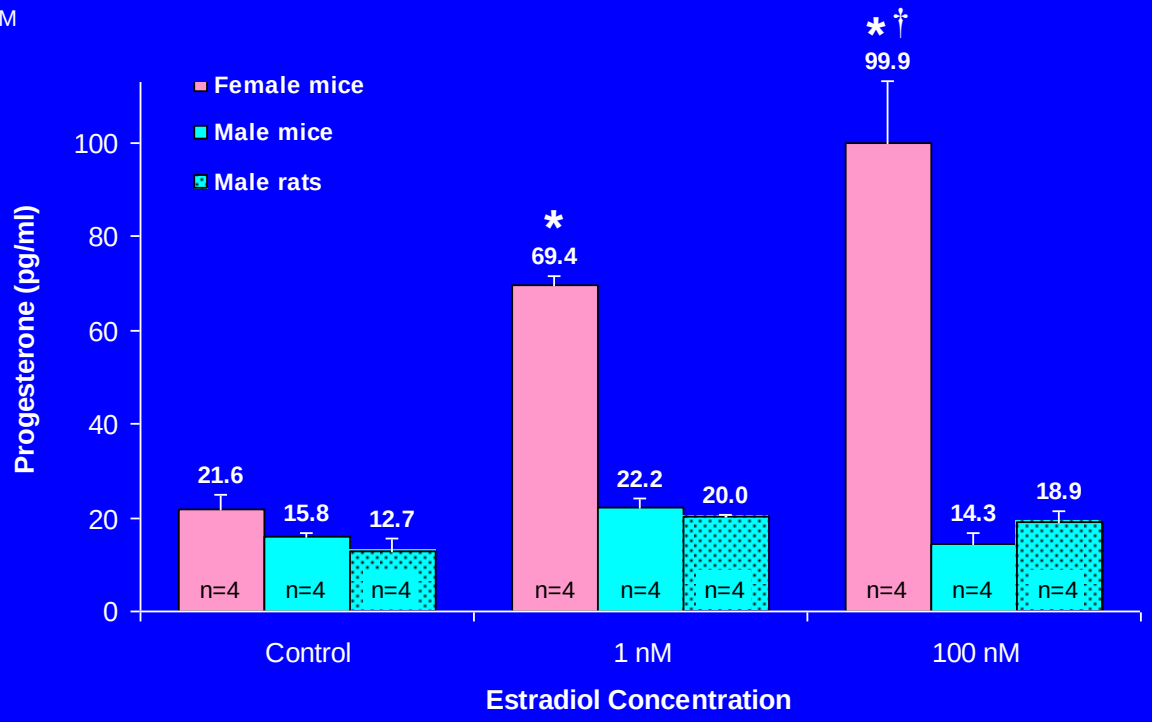
- The estradiol-induced $[Ca^{2+}]_i$ release was abolished, supporting estradiol signaling through mERα, consistent with lack of estrogen positive feedback and sexual receptivity in ERKO mice.
- DPN (100 nM) did not stimulate $[Ca^{2+}]_i$ release, suggesting that DPN acts upon mERα, not mERβ.
- STX induced a similar $[Ca^{2+}]_i$ release compared to wild-type mice, suggesting a signaling pathway independent of mERα. Unclear physiological relevance and function.
- The G-1 induced $[Ca^{2+}]_i$ release was not blocked, supporting G-1 action upon mGluR1a on the plasma membrane and intracellular GPR30.

Overall, mERα is mainly responsible for the rapid, estradiol signaling in hypothalamic astrocytes involved in estrogen positive feedback and the LH surge.

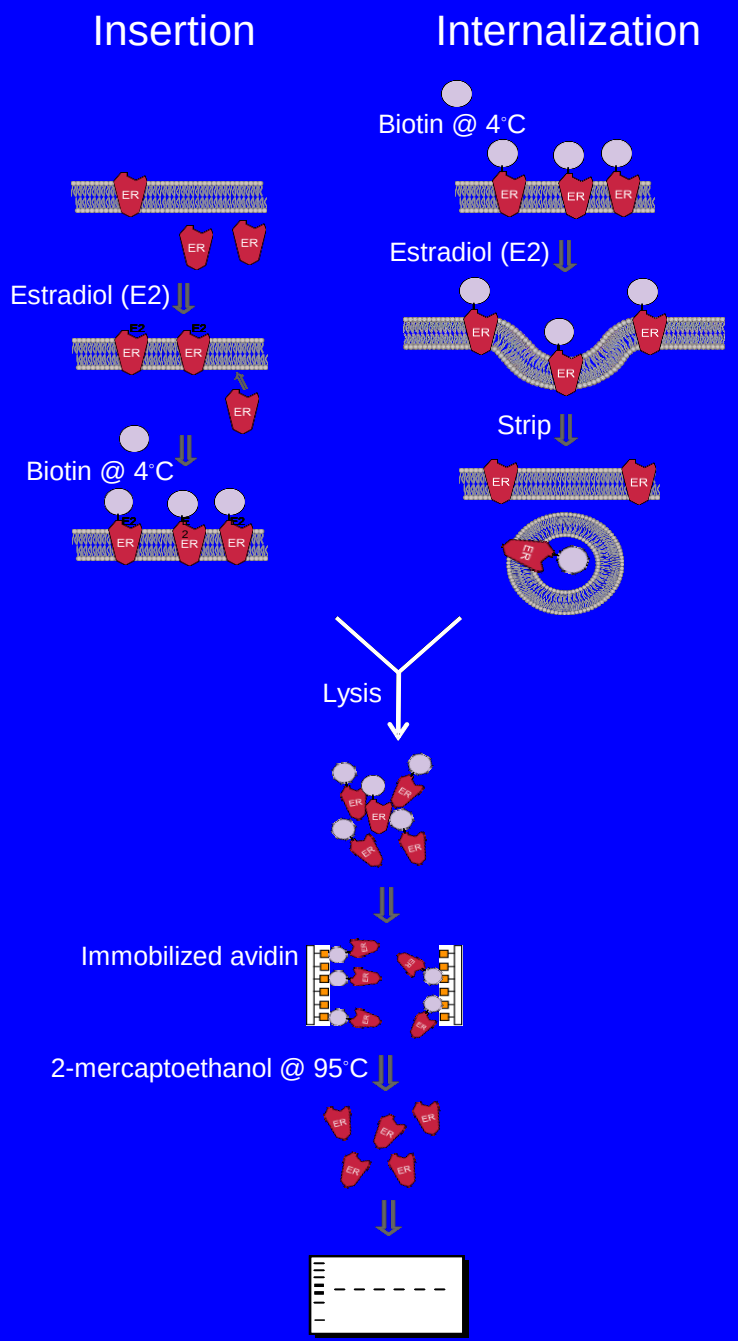
Sex differences in hypothalamic astrocytes.



Kuo et al., ***Biol Sex Differ*** 2010



Surface Biotinylation



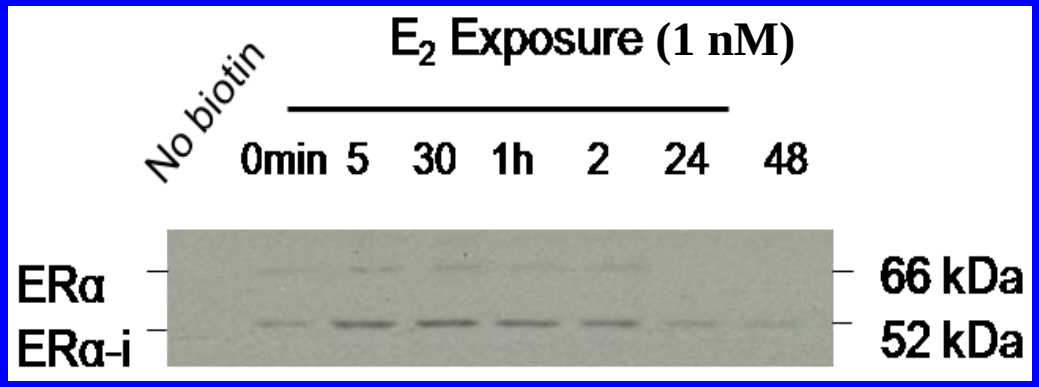
Sulfo-NHS-Biotin:

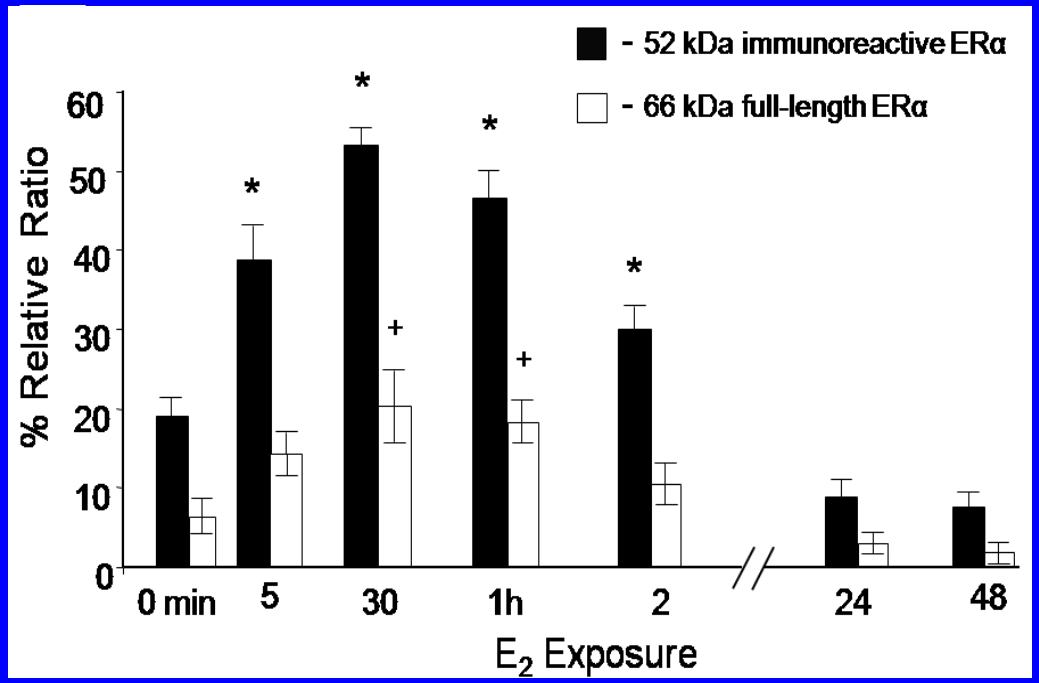
- Water-soluble.
- Membrane impermeable.
- Reacts with primary amines in amino acids and the N-terminus to form stable amide bonds.

Purpose:

- Does mERα have an extracellular portion?
- Does estradiol regulate membrane ERα insertion & internalization?

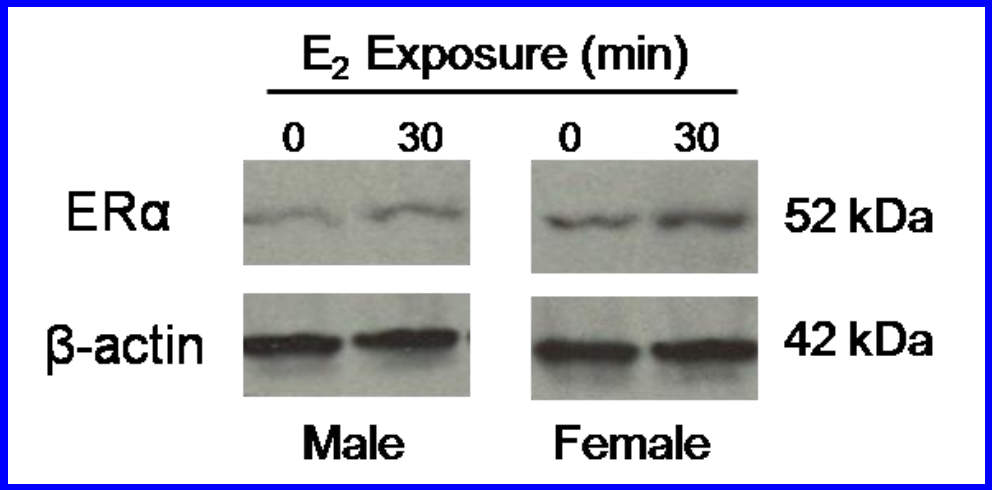
Estradiol stimulation increases, then decreases ERα concentration in the plasma membrane.

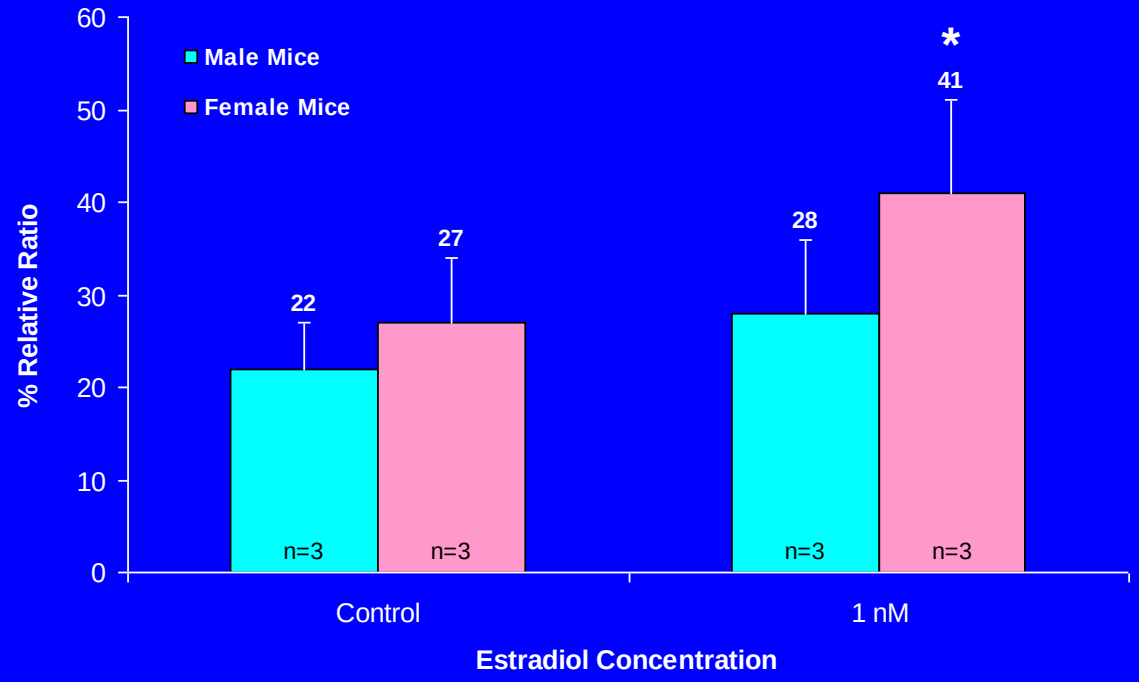




Bondar, Kuo, Hamid & Micevych, ***J Neurosci*** 2009

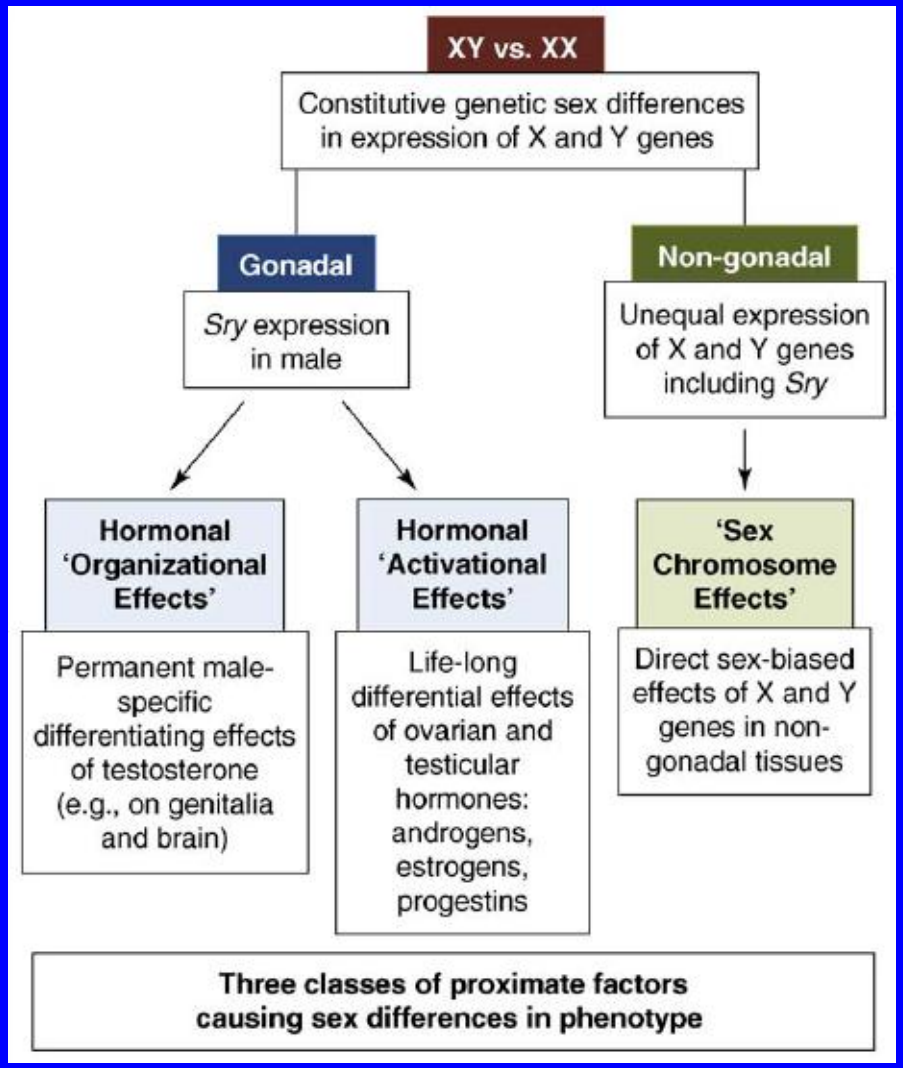
Sex difference in estradiol stimulated membrane ERα insertion.





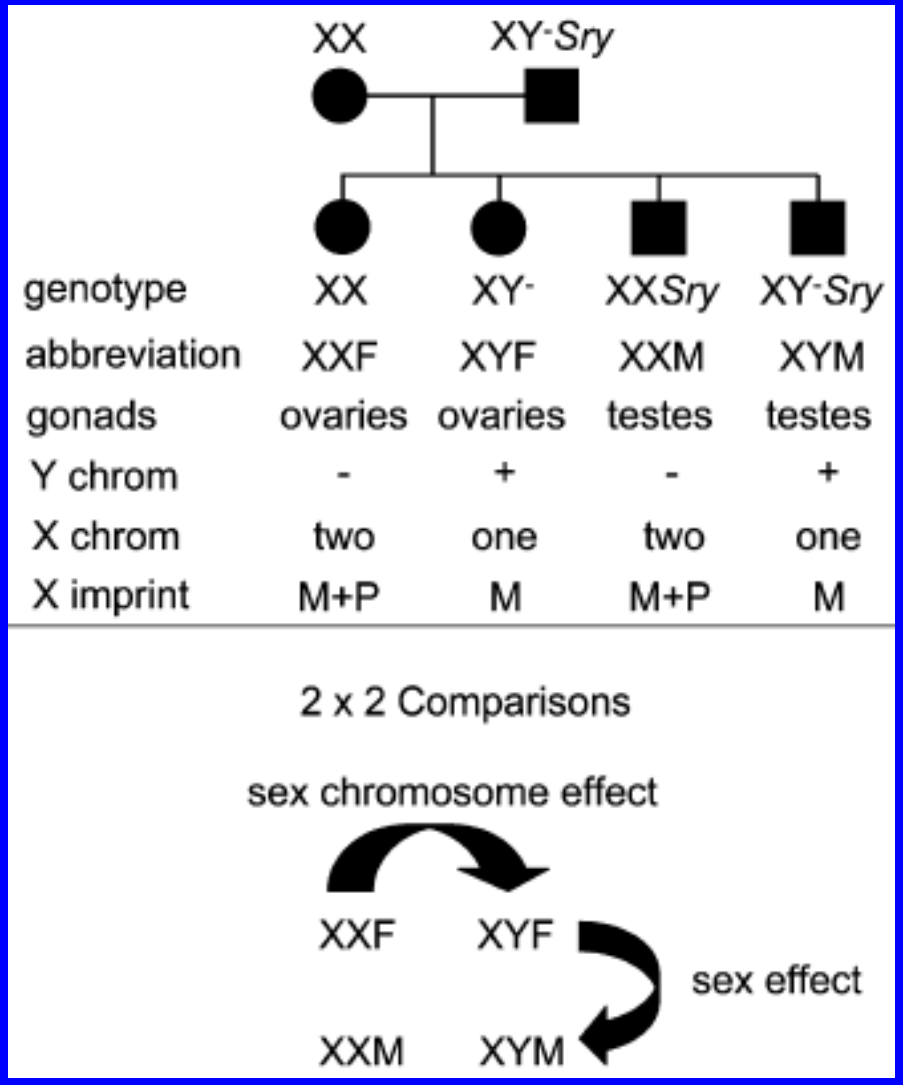
Kuo et al.,
Biol Sex Differ 2010

Sex Differences due to Hormonal and/or Chromosomal effects



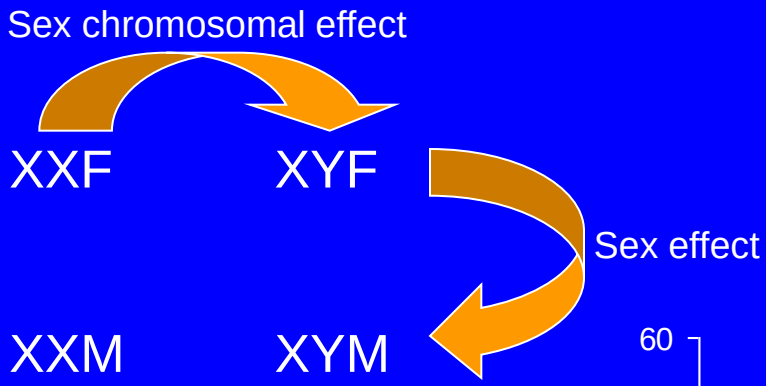
Arnold et al., ***Trends Endocrinol Metab*** 2009

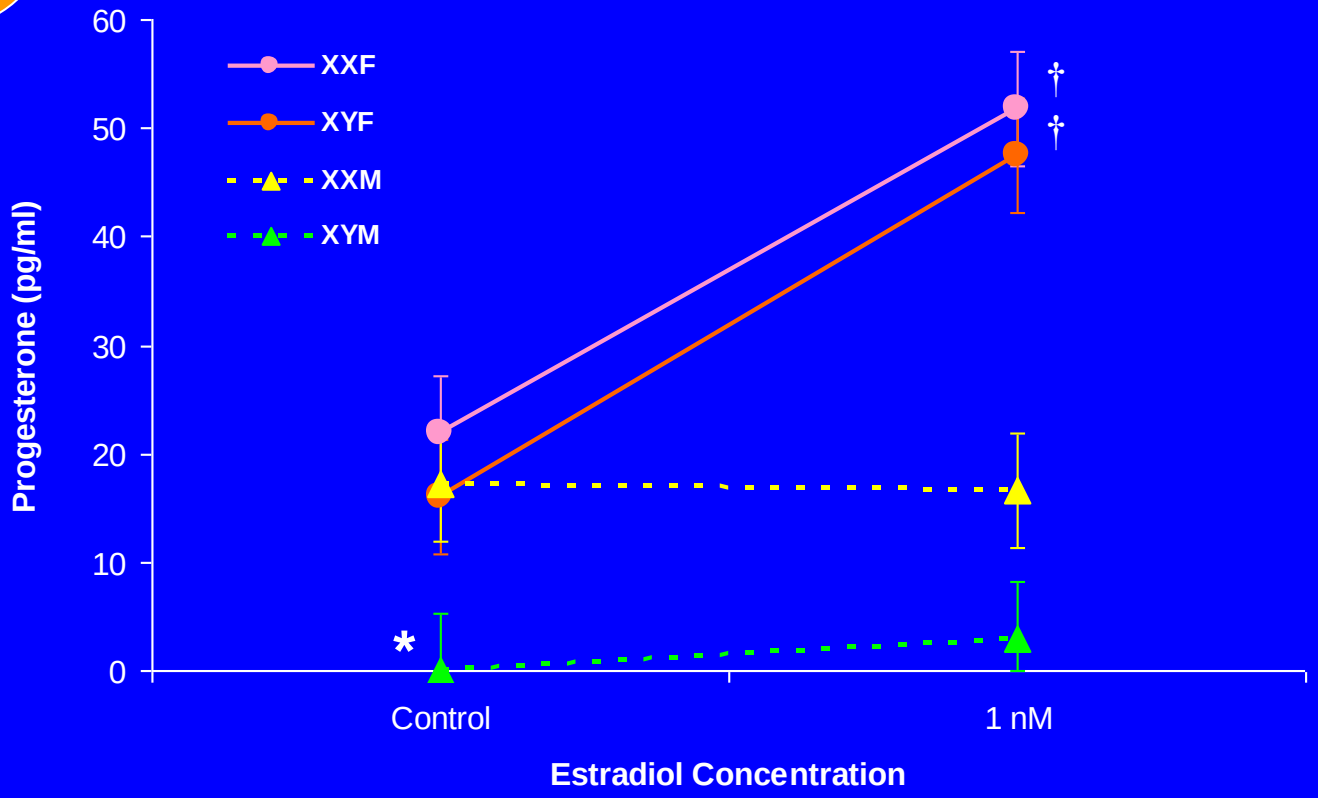
Four Core Genotype Mice



Arnold and Chen, ***Front Neuroendocrinol*** 2009

Sex differences due to gonadal hormone secretion.





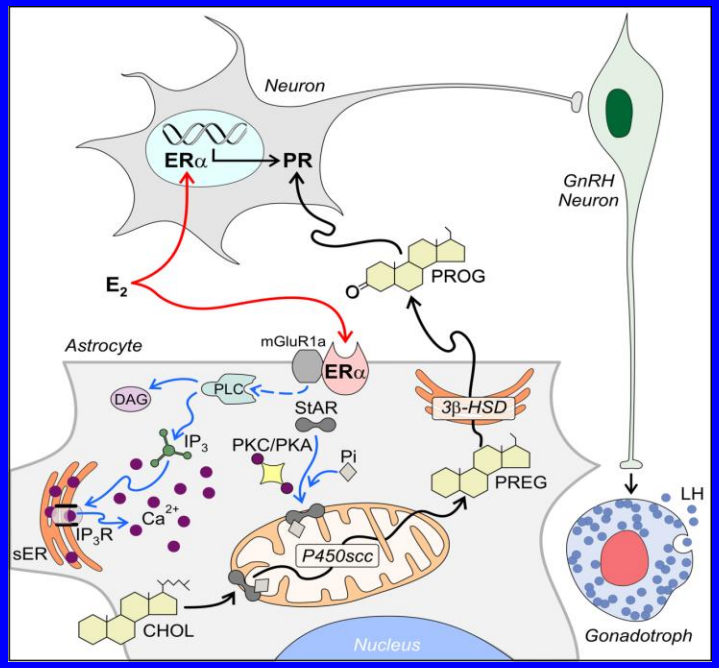
Kuo et al., ***Biol Sex Differ*** 2010

Summary of Findings

- Estrogen positive feedback occurs in female rodents, but not in males. Astrocytic sex differences in estradiol stimulated $[Ca^{2+}]_i$ elevation & progesterone synthesis could explain this difference.
- mERα is an integral membrane receptor with an extracellular portion (not associated with the inner leaflet).
- Sex differences in mERα trafficking could contribute to the greater estradiol responses in female astrocytes.
- These astrocytic sex differences are most probably due to differential secretion of gonadal hormones.

Conclusions

- ERα is an integral membrane receptor and the main mER involved in female reproduction.
- mERα transactivates mGluR1a for rapid cell signaling, leading to increased $[Ca^{2+}]_i$ and progesterone synthesis in hypothalamic astrocytes.



- Hypothalamic astrocyte response to estradiol stimulation at physiologically relevant levels may explain the mechanism for regulation of estrogen positive feedback and the LH surge.
- Astrocytic sex differences may be the key to estrogen positive feedback occurring exclusively in female rodents and most probably result from the differential exposure to gonadal hormones during early development.

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