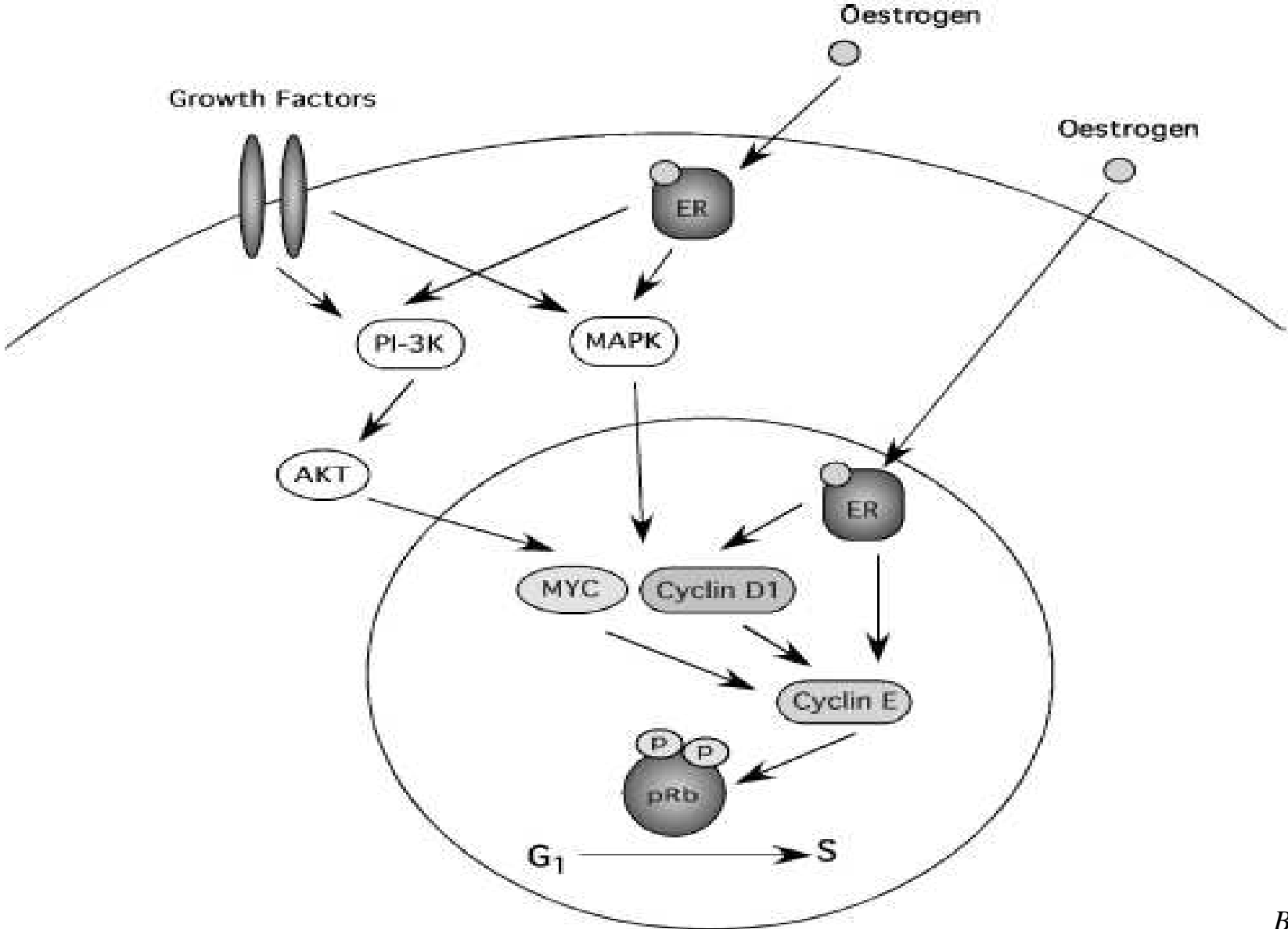




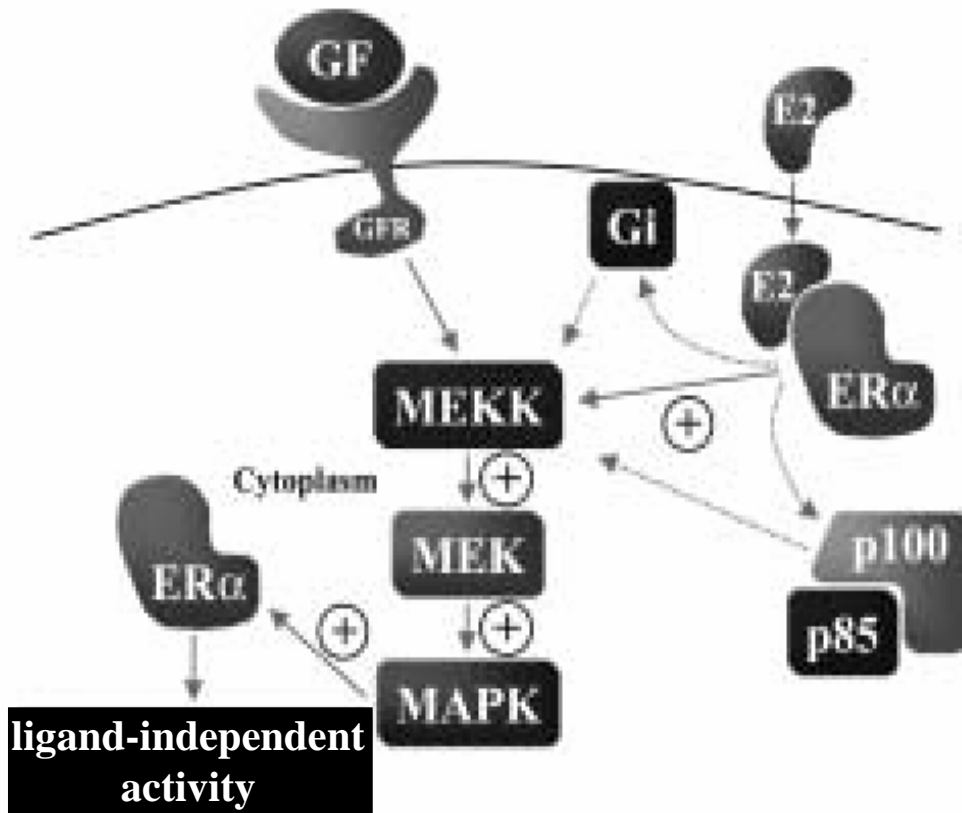
*The best for the brain*

*Estrogens*

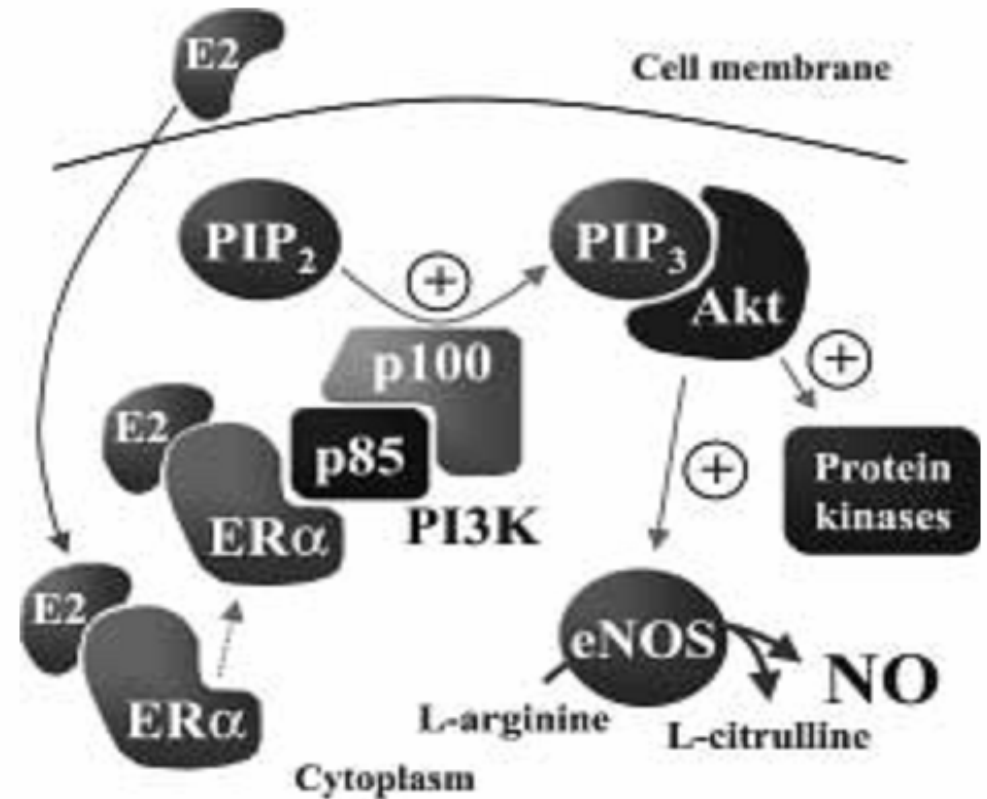
# Cross-talk between growth factor and estrogen pathways



# Cross-talk between growth factor and estrogen pathways

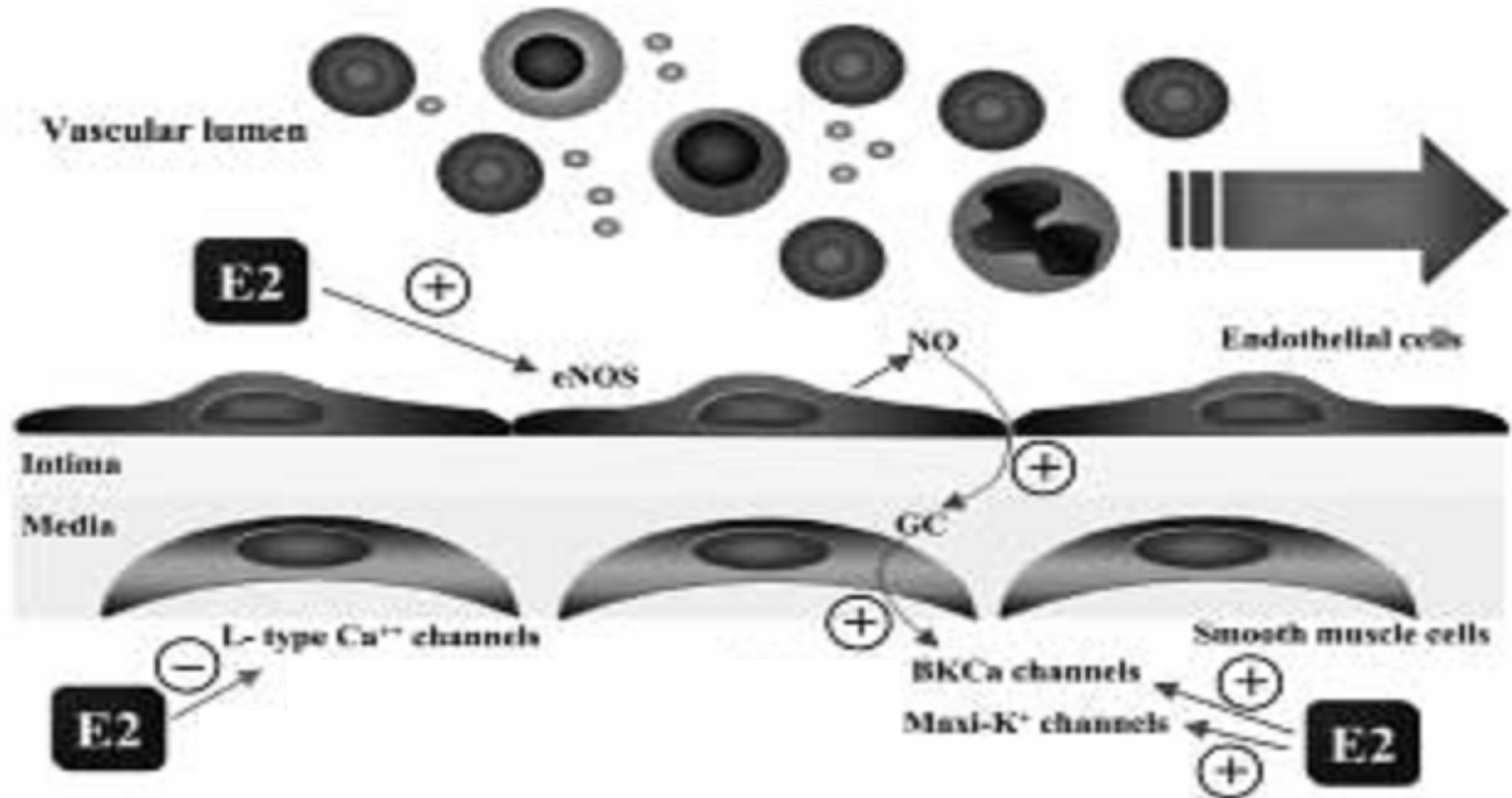


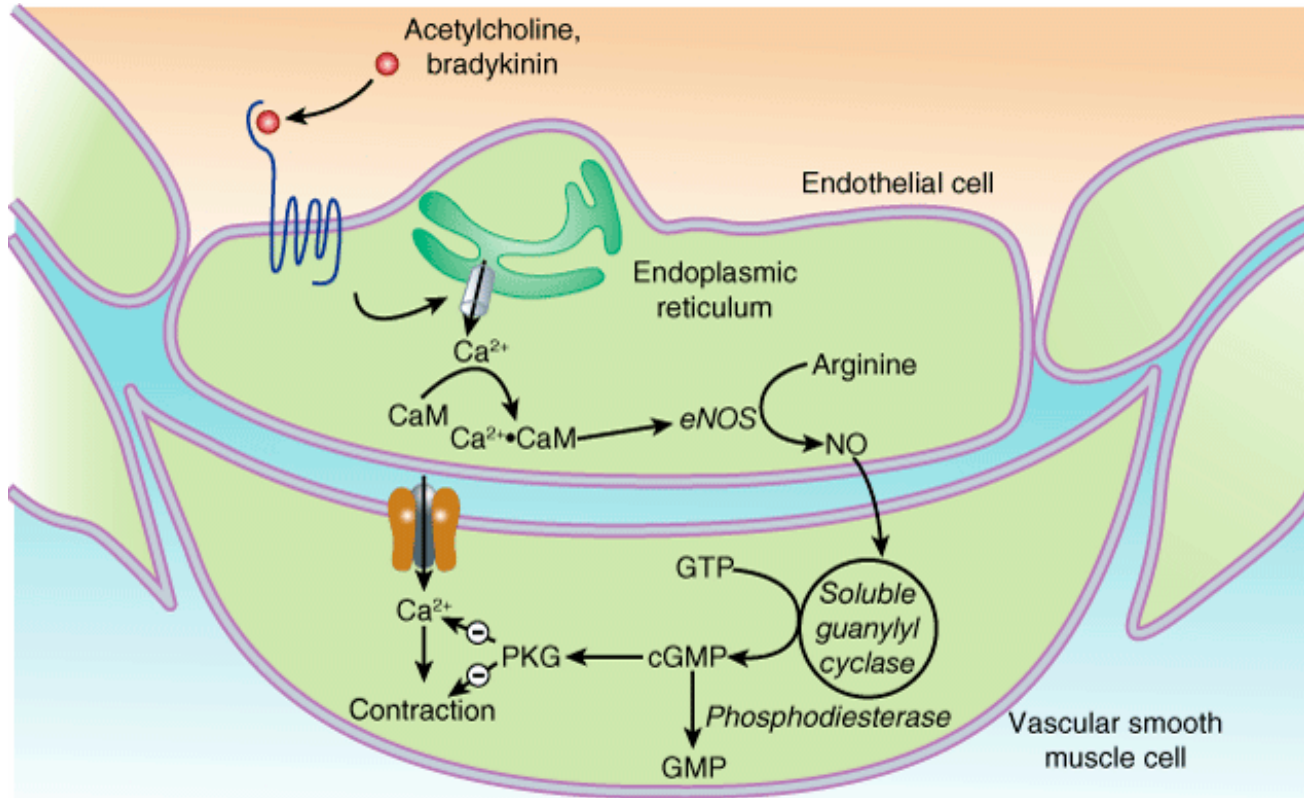
ERs and MAPKs. Binding of growth factors (GF) to the specific membrane receptors induces activation of the MAPK cascade (MEKK → MEK → MAPK). Active MAPK phosphorylates and activates ER independently of the presence of E<sub>2</sub>. On the other side, E<sub>2</sub>-bound ER triggers the activation of the three-step ERK 1/2 MAPK module. ER activation of MAPK is finely modulated by Gi protein (Gi)-dependent and PI3K (p85/p100)-dependent intracellular pathways.



ER and PI3K. Ligand (E<sub>2</sub>)-bound ER interacts with the regulatory subunit (p85) of PI3K (p85/p110). The catalytic subunit (p110) is thus activated, leading to phosphorylation of phosphoinositides (PIP<sub>2</sub>, PIP<sub>3</sub>) on the D-3 position of the inositol ring. PIP<sub>3</sub> is recognized by specific docking sites on protein kinases, which trigger the activation of protein kinase Akt. Akt regulates, via phosphorylation, several intracellular enzymes (protein kinases), including eNOS, leading to increased NO synthesis.

# Estrogen leads to vasorelaxation





**NO has a significant effect on vascular smooth muscle tone and blood pressure - it is a vasodilator.**

**NO protects against thrombosis and atherogenesis (through the inhibition of proliferation and migration of vascular smooth muscle, and inhibition of platelet aggregation).**

**NO may have an additional inhibitory effect on blood coagulation by enhancing fibrinolysis.**

Source: Katzung BG, Masters SB, Trevor AJ: *Basic & Clinical Pharmacology*, 11th Edition: <http://www.accessmedicine.com>

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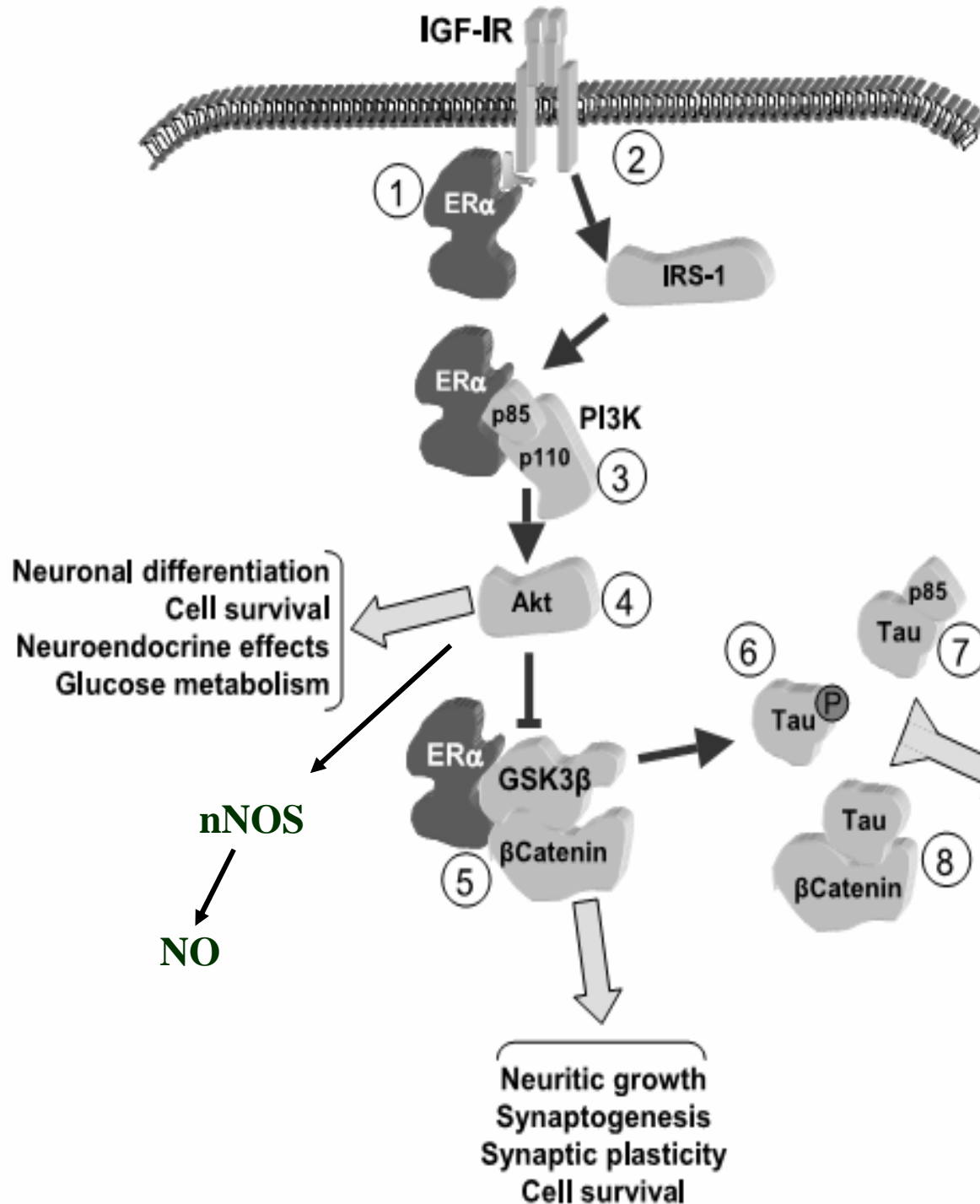
**NO reduces endothelial adhesion of monocytes and leukocytes, due to the inhibitory effect of NO on the expression of adhesion molecules on the endothelial surface.**

**NO may act as an antioxidant, blocking the oxidation of low-density lipoproteins and thus preventing or reducing the formation of foam cells in the vascular wall.**

# Neuroprotective effects of estrogens

## Evidence in vitro

- \* Estrogens in vitro **upregulate expression** of different neurotrophic factors and their receptors (e.g. **BDNF** – brain derived neurotrophic factor, **NGF** – neurone growth factor).
- \* Estrogens **increase expression** of structural proteins of neurons (proteins in neurofilaments and microtubules, **TAU**, **GAP43** – proteins necessary for growth of axons) and **activation of nNOS**.
- \* Hyperphosphorylation of TAU leads to TAU dysfunction and is one of the basic pathogenic mechanisms in Alzheimer disease. Both in vitro and in vivo the **kinase which phosphorylates TAU** (glycogen kinase 3 $\beta$ , **GSK3 $\beta$** ) is inhibited by estrogens.



## Estradiol in the brain:

- induces the association of ERα with IGFIR

- increases IGFIR phosphorylation

- modulates interaction between ERα and p85 (PI3K)

- increases Akt phosphorylation

- decreases GSK3β thereby decreases Tau phosphorylation

- increases interaction of p85 and βcatenin with Tau.

# Neuroprotective effects of estrogens

## Evidence in vivo

- Rat females are much less sensitive to brain ischemia, trauma or some neurotoxins than males. Also in human the frequency of stroke and reperfusion injury is much rarer in premenopausal women than in men at the same age.
- Expression of aromatase and production of estrogens is induced in the glial cells in response to injuries.
- Estrogens delivered just before or soon after (up to 3 h) after trauma is neuroprotective (data from rats).
- Estrogens reduce reperfusion induced brain injury via:
  - \* upregulation of Bcl2.
  - \* upregulation of caspase inhibitors. } inhibition of apoptosis
- Estrogens can act as a neuronal growth factor. Rat female have more new formed neurons in hippocamp than males at the same age.



# Neuroprotective effects of estrogens

## Clinical and epidemiological evidence

- Decrease in estrogen level leads to decrease in verbal memory
- Neurodegenerative diseases have lower frequency, later time of onset, slower progression, and better prognoses in premenopausal women than in men. After menopause these differences disappear.
- Protective effect of estrogens in premenopausal women was proposed in:
  - \* schizophrenia
  - \* Alzheimer Disease (AD)
  - \* Parkinson Disease (PD)
  - \* Stroke
- Possibly, low levels of estrogen can be a risk factor in AD, while high expression of one of ER $\beta$  isoform may play a protective role. Data, however, are still not conclusive.

**Estrogen's role in memory may be due to its effect on the tiny knobs called spines that protrude from a neuron's dendrites. Spines are formed at the site of connections between neurons.**

**The images demonstrate that estrogen resulted in a two-fold increase of the number of spines in rat embryo brain cells compared to those without the supplements of estrogen.**

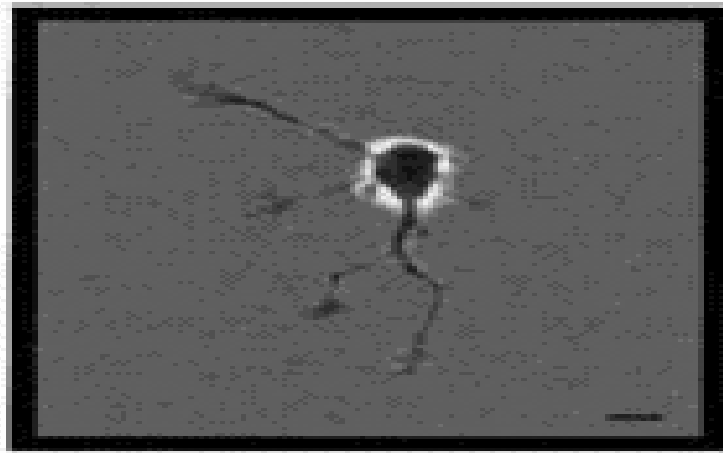
with estrogen



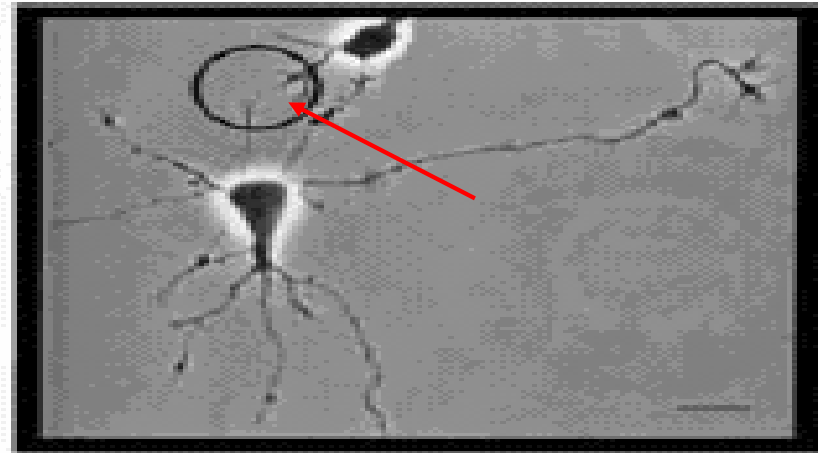
without estrogen



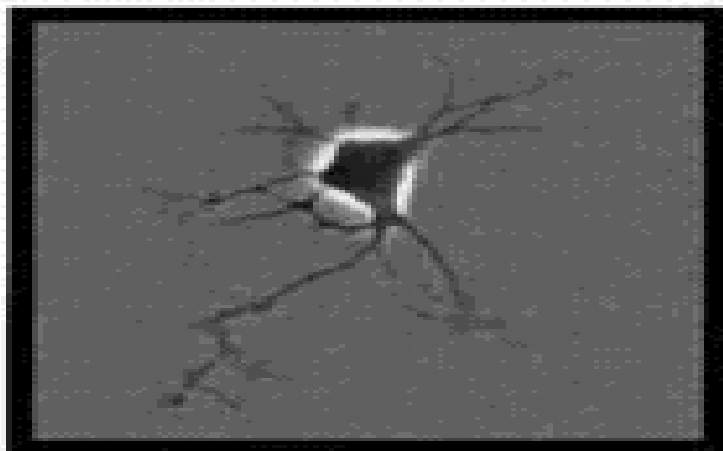
# Effect of estrogen on hippocampal neurons in vitro



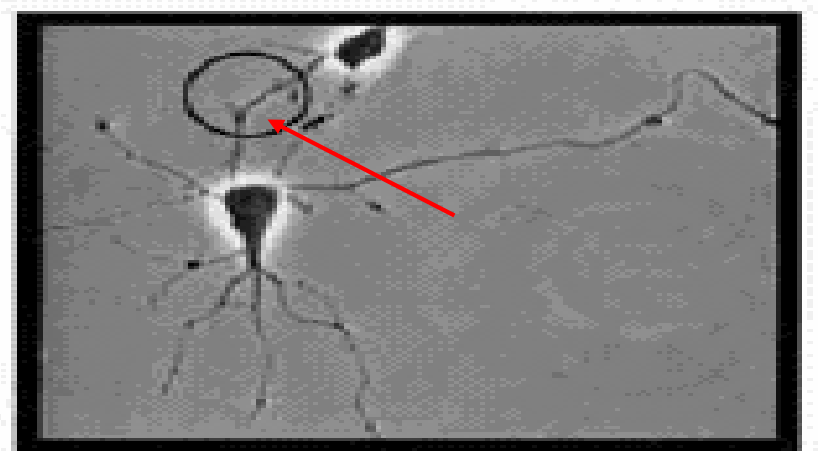
**Control**



**Prior to CEE**



**Following 24 hr  
CEE exposure**



**Following 1 hr CEE  
exposure**

*CEE- conjugated equine estrogen (used in HRT)*

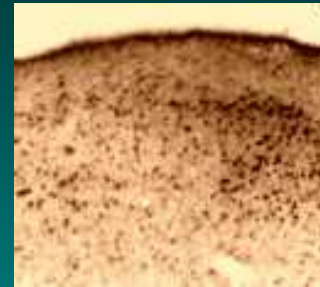
*R.D. Brinton et al. / Neurobiology of Aging 21 (2000) 475–496*

# Mechanisms of neuroprotective activity of estrogens:

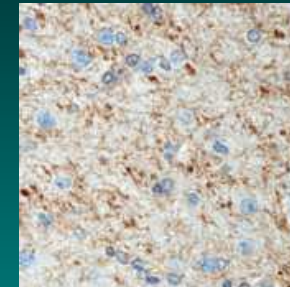
## Genomic (classical) pathway:

\* Slow - effects after several hours

\* Mediated by ER $\alpha$  and ER $\beta$



*ER $\alpha$  - amygdala*



*ER $\beta$  - cortex*

\* ER are expressed in different cells of central and peripheral nervous system. In the brain, in many cell types both types of receptors are coexpressed. Possibly both are involved in neuroprotection.

\* Genes with ER response elements in the promoter:

# oxytocin (peptide hormone), apoE (neuroregeneration, clearance of  $\beta$ -amyloid)

# somatostatin (peptide hormone)

# BDNF, NGF, and their receptors

# TGF $\alpha$  (transforming growth factor- $\alpha$ )

# IGF-I (anti-apoptotic protein)

# BCL-2 (anti-apoptotic protein)

# choline acetyltransferase (produces ACh)

# tyrosine hydroxylase (produces dopamine)

# Mechanisms of neuroprotective activity of estrogens:

## Non-genomic pathway (influence on other signal transduction pathways):

\* Can be very fast – effects after minutes.

\* mediated by:

# activation of adenylyl cyclase, elevation of cAMP, induction of PKA, CREB, and AP-1

# elevation of  $Ca^{2+}$  and stymulacja PLC

# activation of PI3K and Akt kinases (ER $\alpha$  may directly bind to PI3K).

# activation of ERK1/2 (survival signaling).

# activation of Src kinase (and thereby MAP kinases).

\* Activation of **PI3K and Akt kinase** increase activity of **nNOS and eNOS** (neuronal and endothelial nitric oxide synthase) – it can be one of the mechanisms of neuro- and cardioprotection.

\* Inhibition of NF $\kappa$ B attenuates inflammatory response

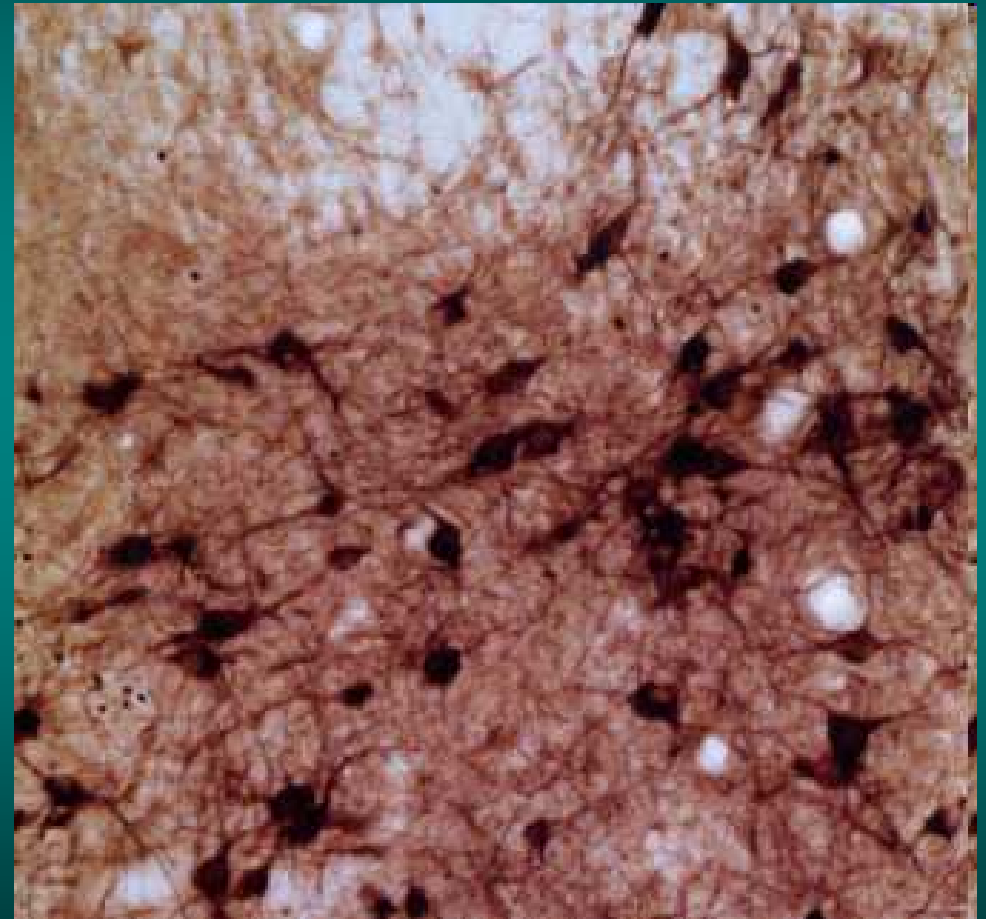
## **NO in the peripheral nervous system:**

- NO act as a mediator of certain nonadrenergic, noncholinergic (NANC) neurons.
- NO is a major mediator of penile erection.

*rat brain - nNOS*

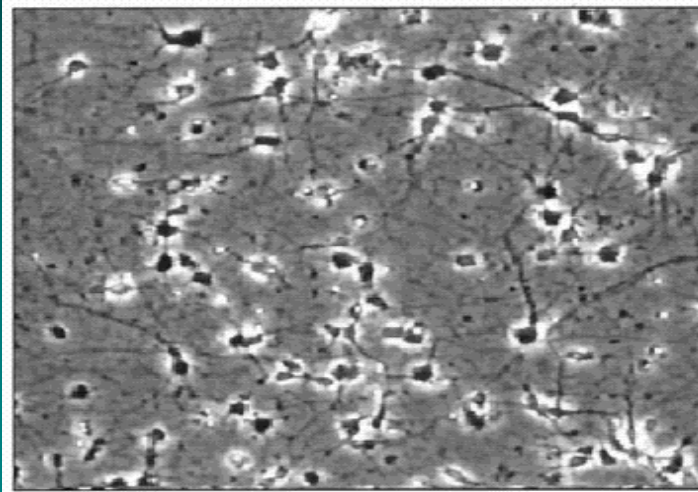
## **NO in the central nervous system:**

- NO is an important neurotransmitter. Unlike classic transmitters NO is not stored, but synthesized on demand and immediately diffuses to neighboring cells. NO synthesis is induced at postsynaptic sites in neurons, most commonly upon activation of of glutamate receptor, which results in calcium influx and activation of nNOS.
- NO synthesized postsynaptically may function as a retrograde messenger.
- NO plays a major role in the regulation of synaptic plasticity, the process of synapse strengthening that underlies learning and memory.

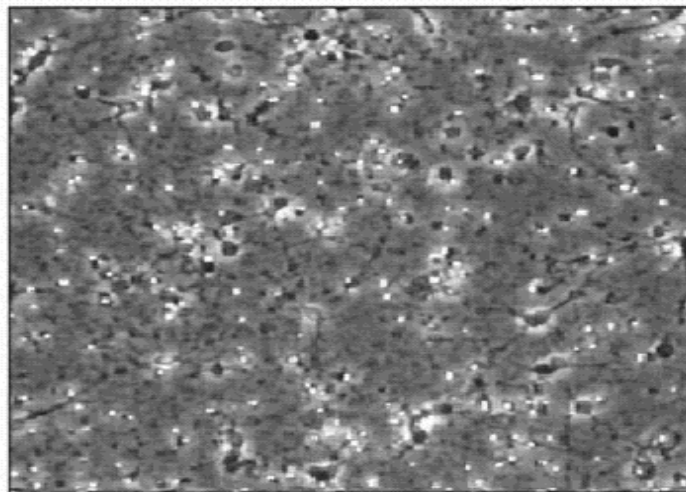


# Neuroprotective effect of CEE against glutamate-induced toxicity

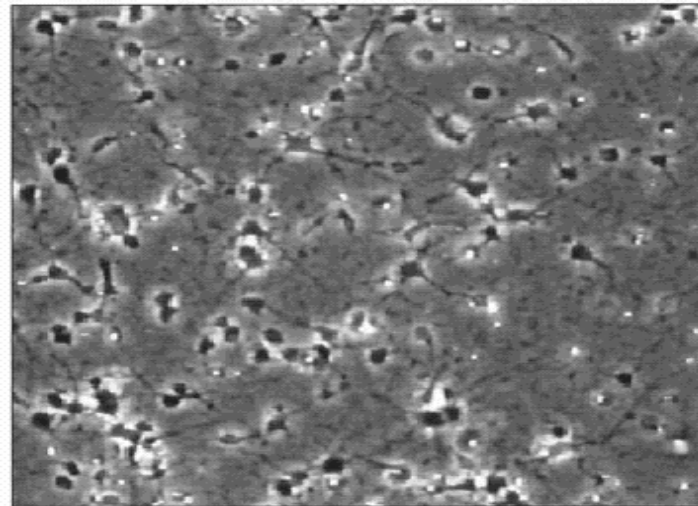
*(Hippocampal neurons cultured in vitro)*



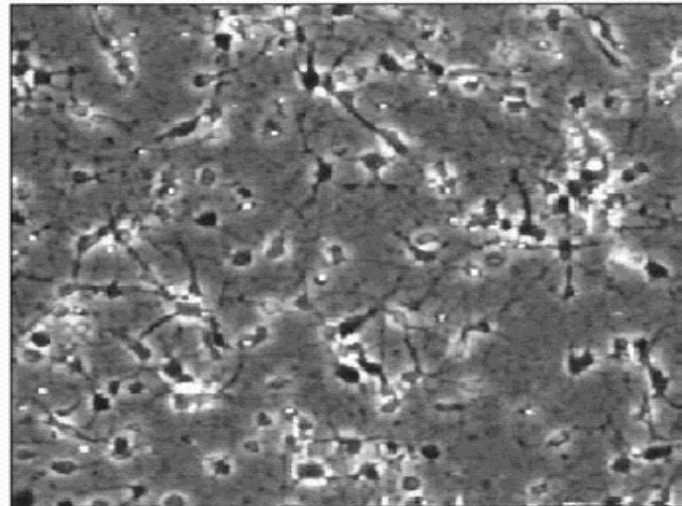
Control



Glutamate



Glutamate + CEE 0.1ng/ml



Glutamate + CEE 10ng/ml

plating the cells

4 days

supplementation with CEE

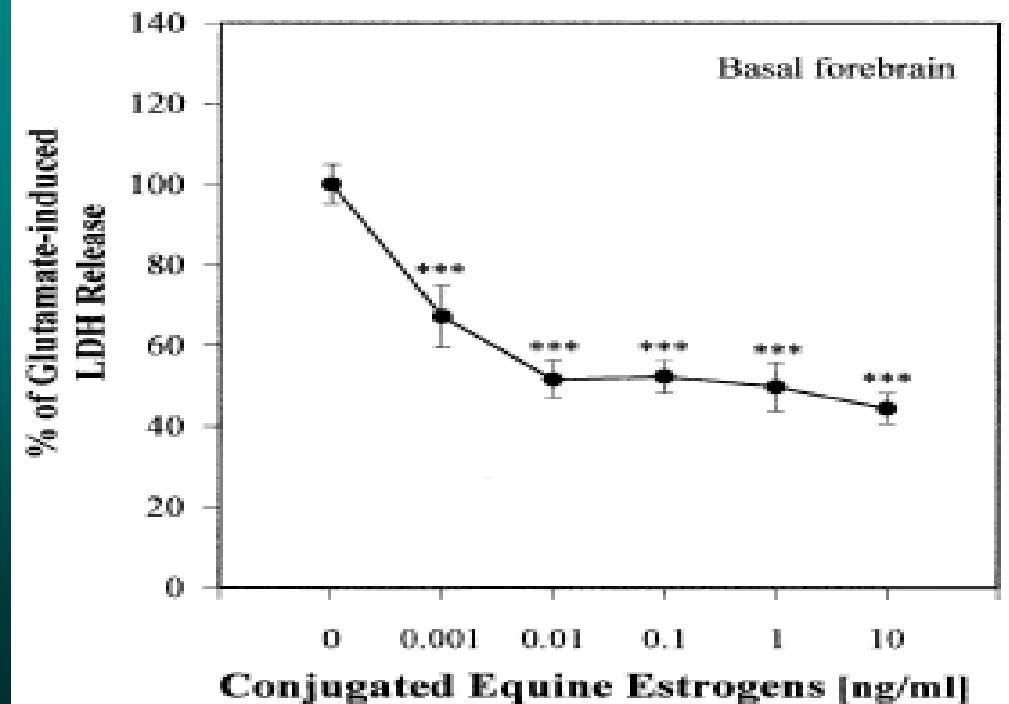
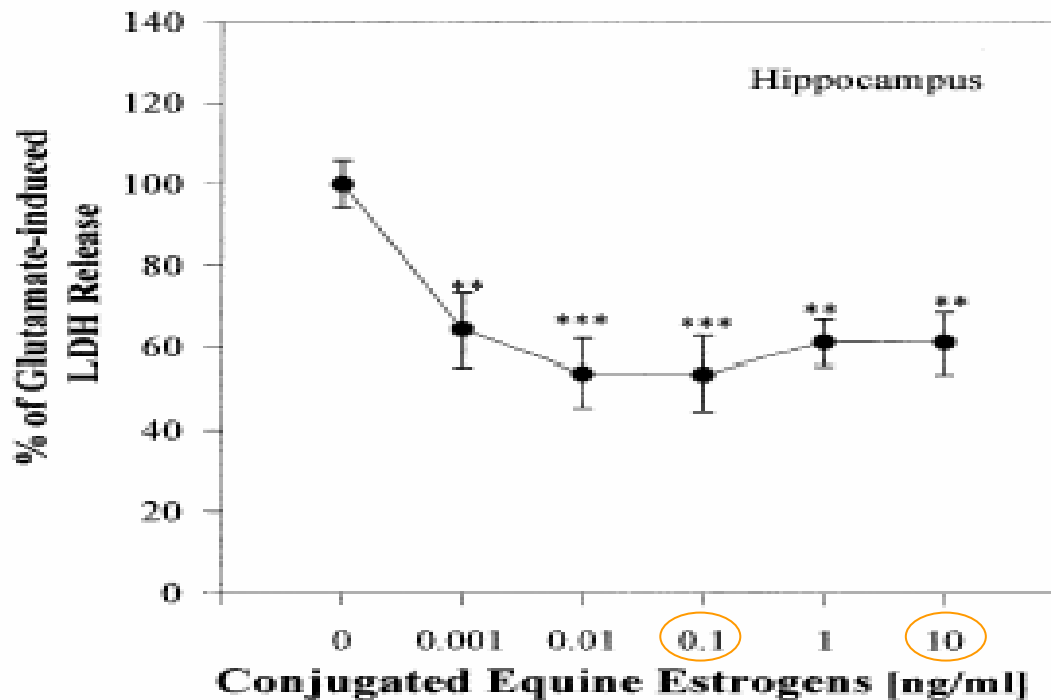
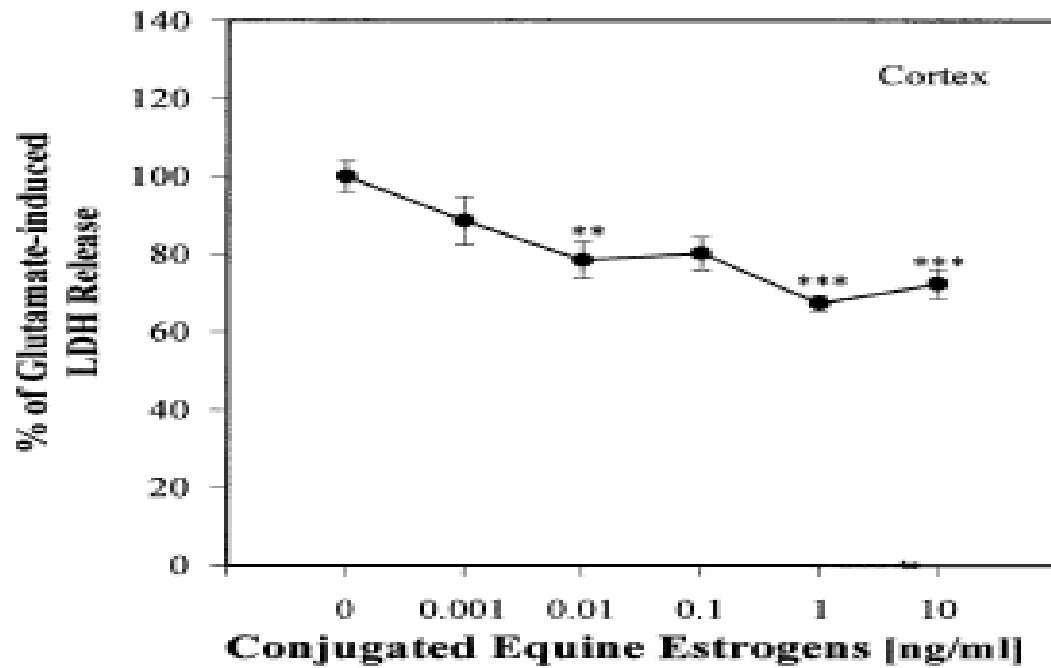
4 days

exposure to glutamate (5 min)

24 h

analysis

## Neuroprotective effect of CEE against glutamate-induced toxicity





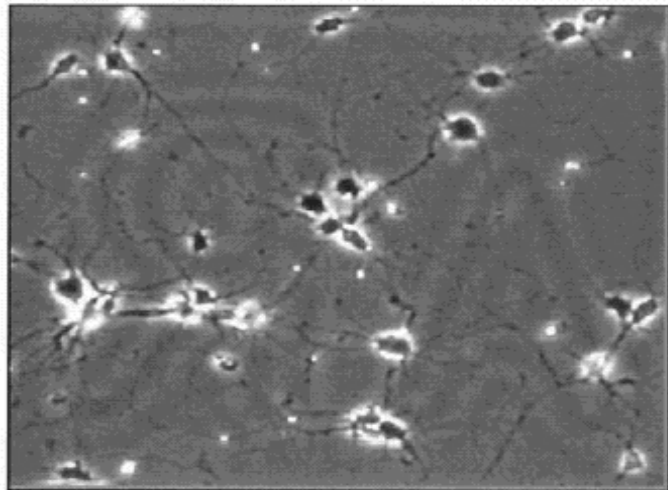
# Mechanisms of neuroprotective activity of estrogens:

## Antioxidative activity:

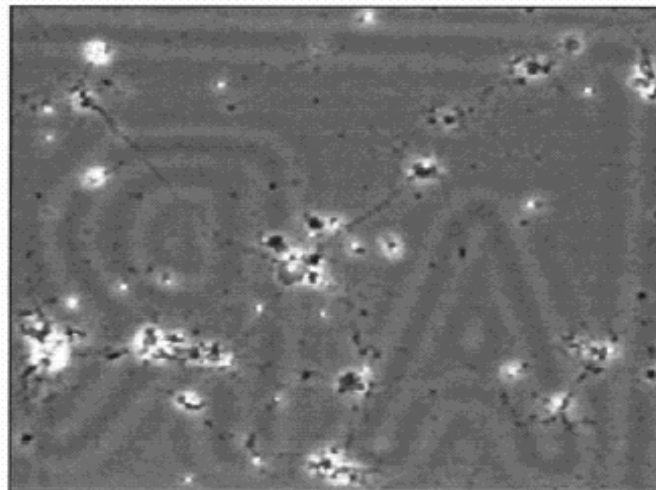
- \* Estrogen has similar activity to vitamin E and may inhibit lipid peroxidation. This effect is independent of ER.
- \* Estrogens protect neurons against  $O_2^-$ ,  $H_2O_2$ , and  $HO\cdot$ .
- \* Effective concentrations of estrogens to obtain antioxidative effect are within micromolar range, much above physiological concentrations. However, the level of estrogens may vary. Although usually the level of estrogens can reach only low nanomolar levels, local accumulation to micromolar concentrations cannot be excluded.
- \* Noteworthy, effective concentration of estrogens may be much lower (within physiological range), e.g. in the presence of glutathione.

# Neuroprotective effect of CEE against H<sub>2</sub>O<sub>2</sub>-induced toxicity

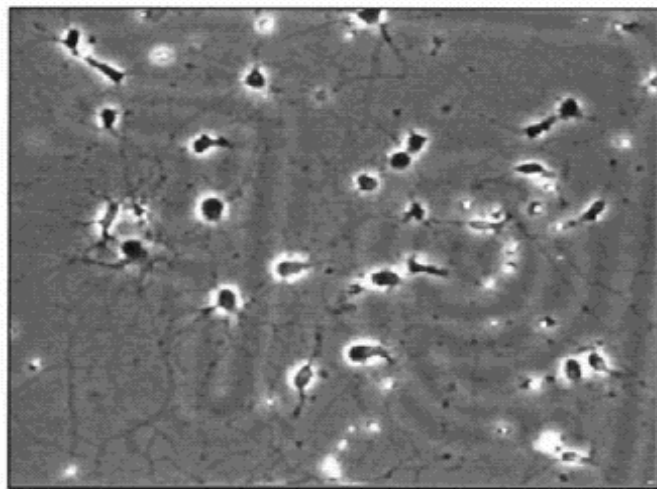
(Hippocampal neurons cultured *in vitro*)



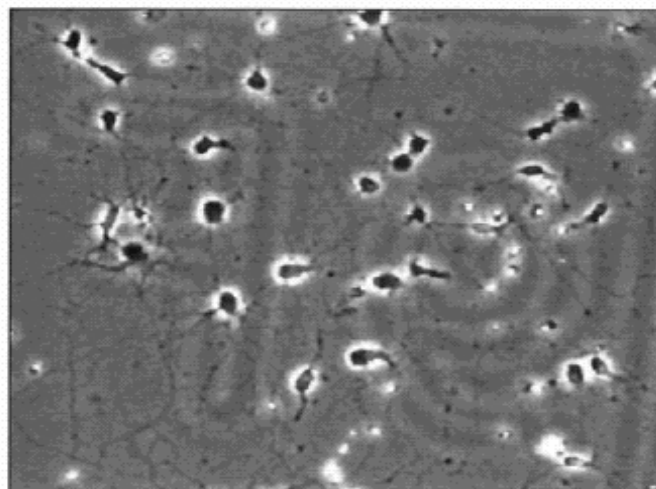
Prior to H<sub>2</sub>O<sub>2</sub>



H<sub>2</sub>O<sub>2</sub>



Prior to H<sub>2</sub>O<sub>2</sub>



H<sub>2</sub>O<sub>2</sub> + CEE 0.1ng/ml

plating the cells

4 days

supplementation with CEE

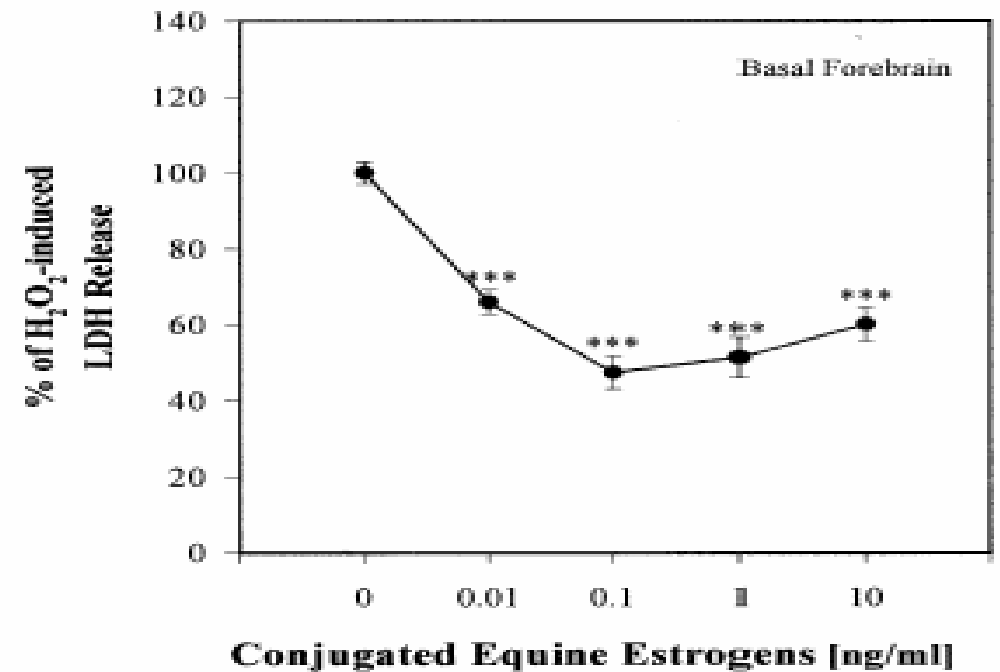
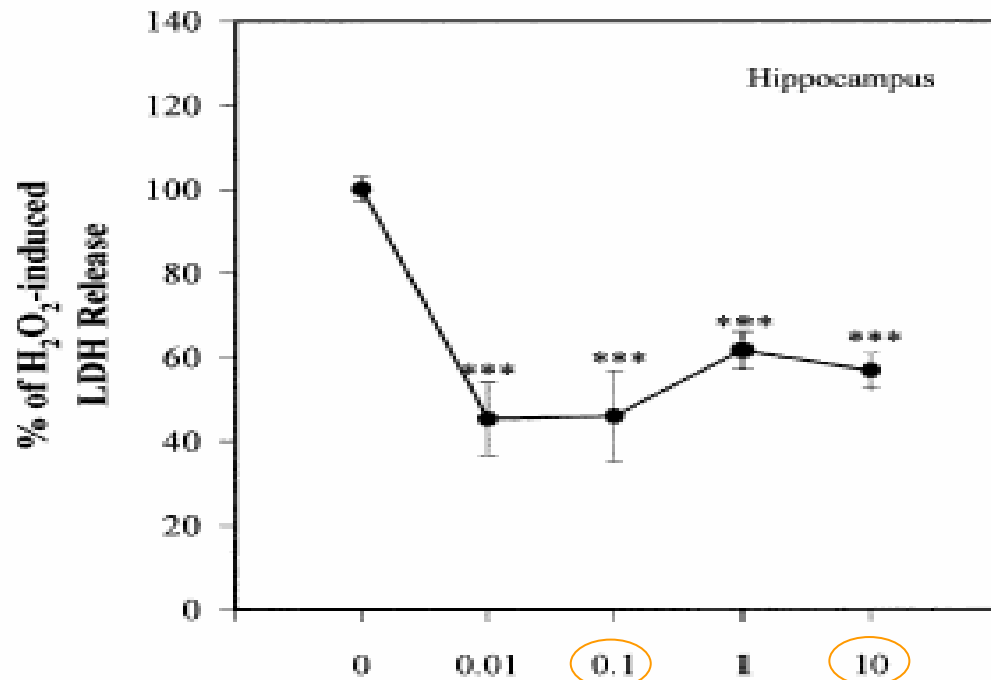
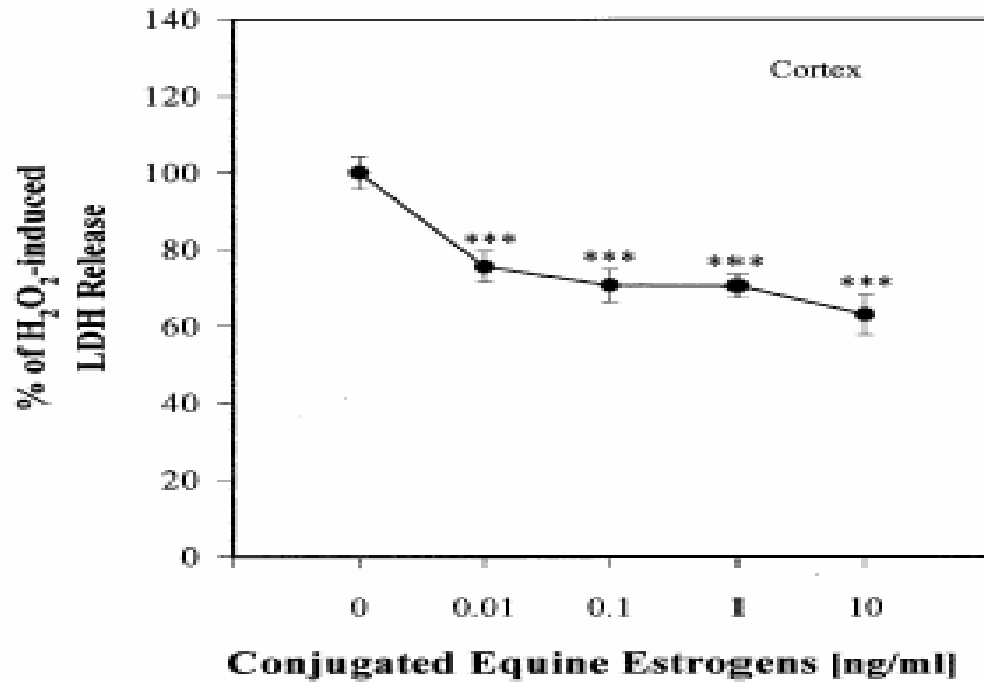
4 days

exposure to H<sub>2</sub>O<sub>2</sub> (20 min)

24 h

analysis

## Neuroprotective effect of CEE against H<sub>2</sub>O<sub>2</sub>-induced toxicity



## Possible Mechanisms of Estrogen/SERM Neuroprotection

### ESTROGEN/SERMs

### Neuroprotection

#### Genomic

- Requires pretreatment
- Involves ER-mediation
- Involves gene transcription

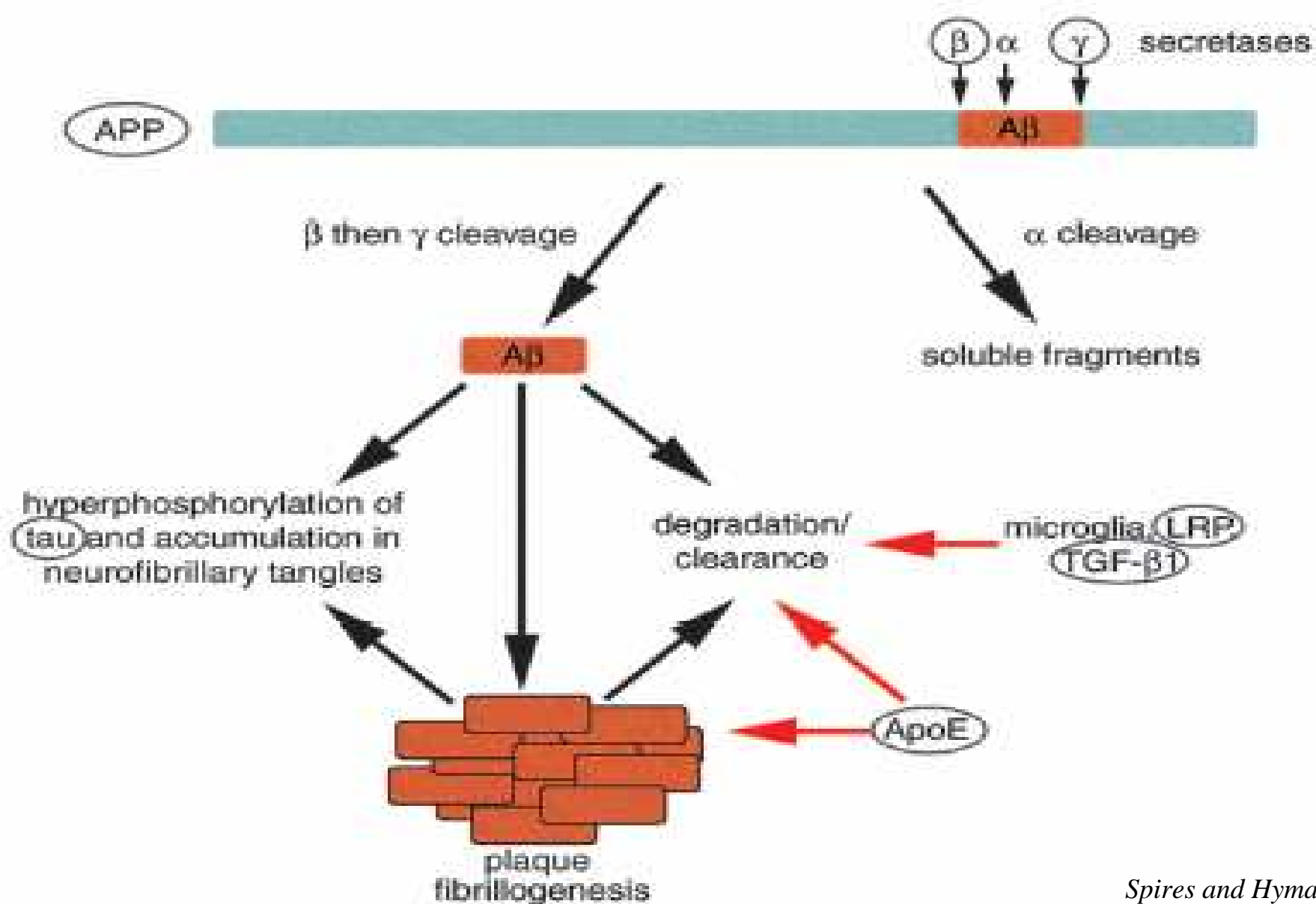
#### Nongenomic

- Rapid effect
- May or may not involve ER-mediation
- May involve MAPK or AKT signaling

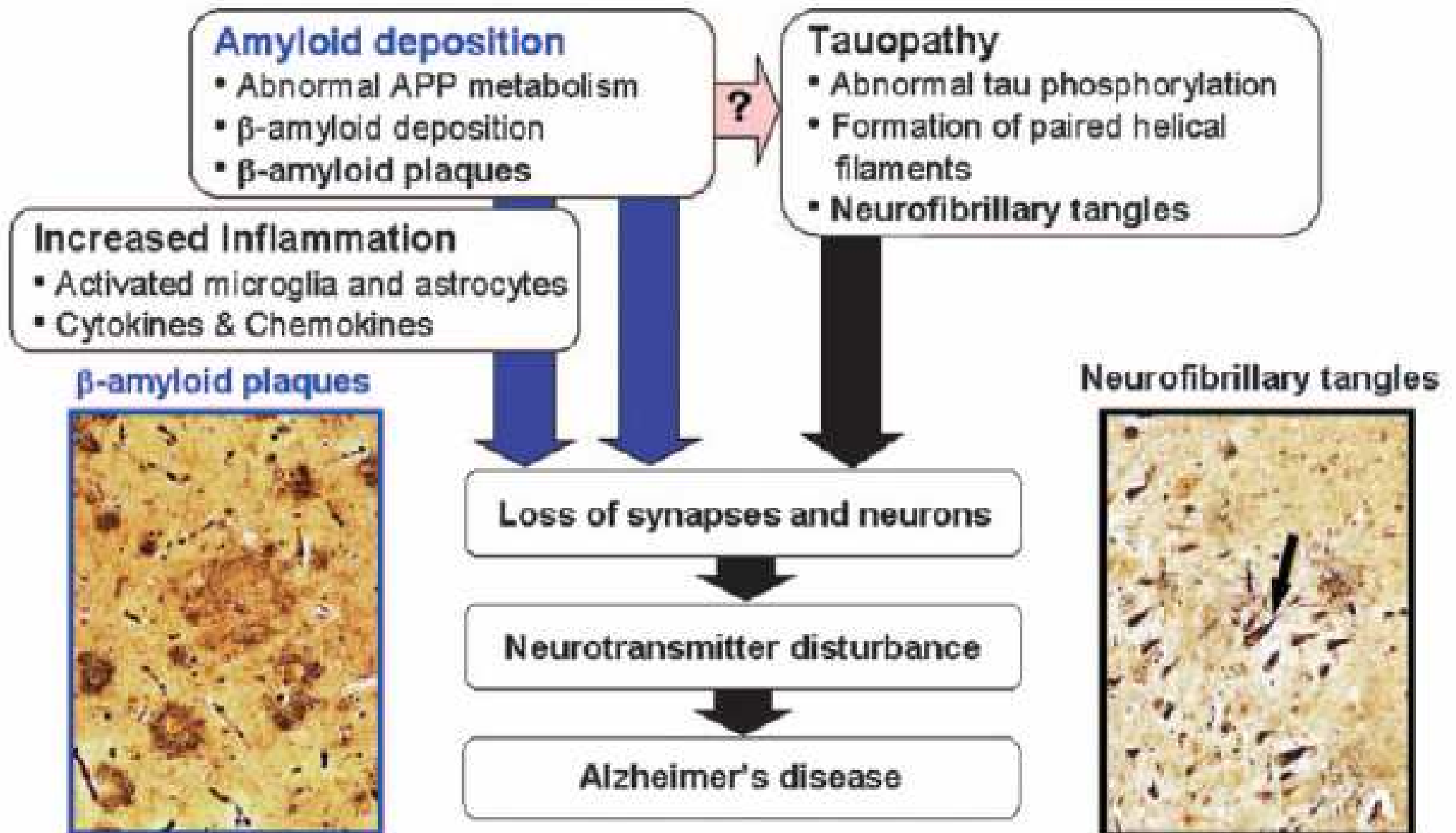
#### Antioxidant

- Scavenges free radical oxidants
- *Does not* involve ER-mediation
- Usually only observed at very high *pharmacological* concentrations

Amyloid precursor protein cleavage by  $\beta$ - and  $\gamma$ -secretases leads to the production of amyloid- $\beta$ , the primary component of senile plaques in AD.



# The neuropathological steps of Alzheimer's disease



# Groups of drugs for treatment of AD:

- **Acetylcholinesterase inhibitors** (e.g. tacrine, donepezil, galantamine)
- **Blockers of NMDA receptors** - major excitatory glutamate receptors in the brain (e.g. memantine)
- Metal chelators (disruption of structure of amyloid- $\beta$ ).
- BACE-1 ( $\beta$ -amyloid secretase-1) inhibitors
- Intrabodies (single-chain antibodies) blocking the  $\beta$ -secretase cleavage site of APP
- $\gamma$ -secretase inhibitors
- active or passive immunization against  $\beta$ -amyloid<sub>1-42</sub> (not only slower progression but also reduction of existing plaques)

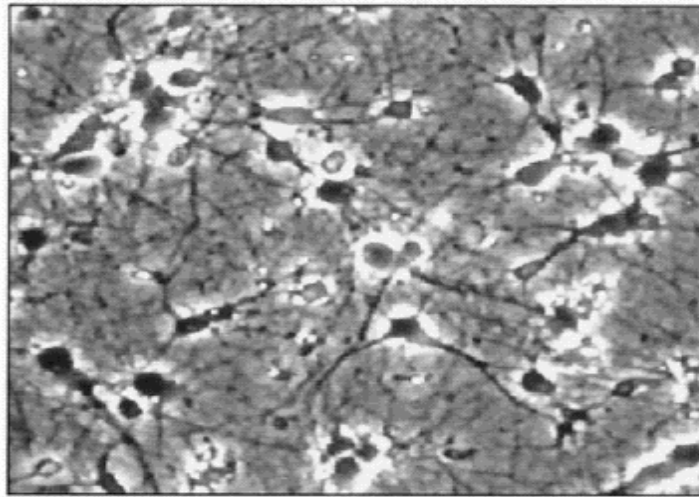
# Neuroprotective estrogens modify multiple aspects of Alzheimer Disease

- decrease the generation and toxicity of  $\beta$ -amyloid
- decrease the damage induced by free radicals generated by  $\beta$ -amyloid
- protects against the secondary insult induced by excessive leakage of glutamate from damaged neurons
- augments expression of anti-apoptotic genes, such as Bcl-2 or caspase inhibitors
- increase expression of choline acetyltransferase

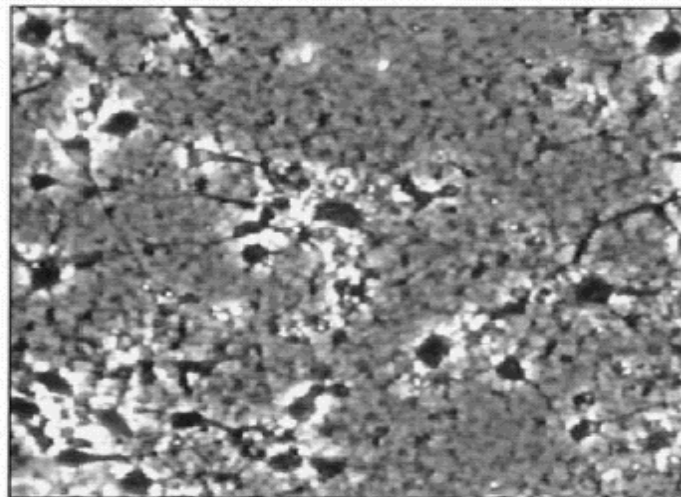


# Neuroprotective effect of CEE against $\beta$ -amyloid-induced toxicity

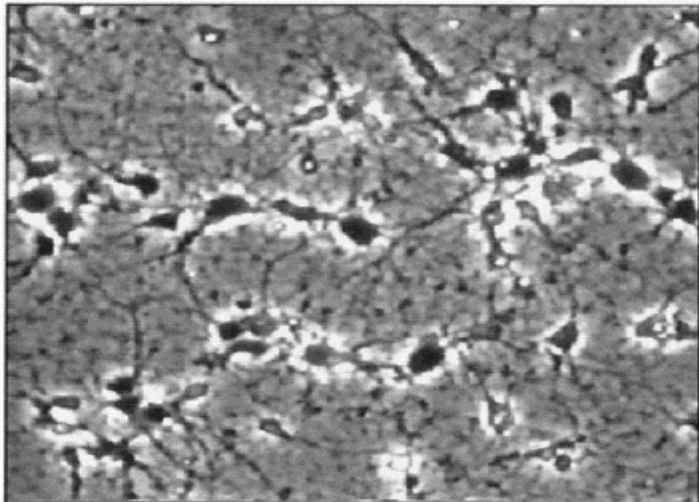
(Hippocampal neurons cultured *in vitro*)



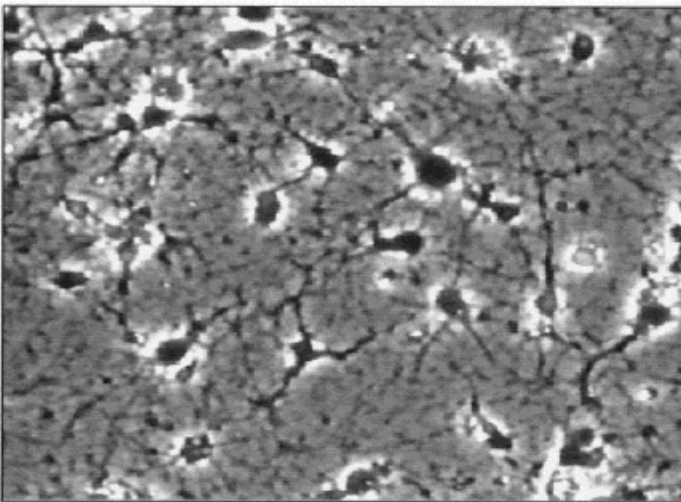
Control



$\beta$ -Amyloid



$\beta$ -Amyloid + CEE 0.1ng/ml



$\beta$ -Amyloid + CEE 10ng/ml

plating the cells

4 days

supplementation with CEE

4 days

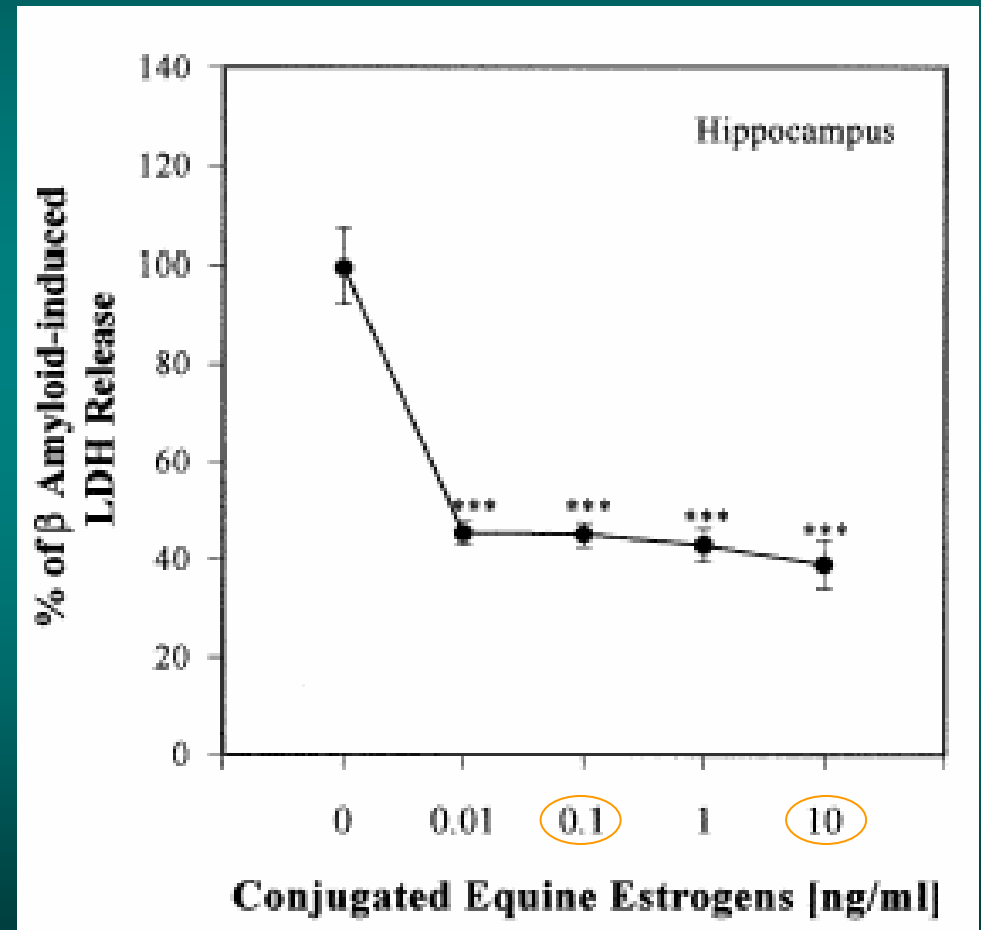
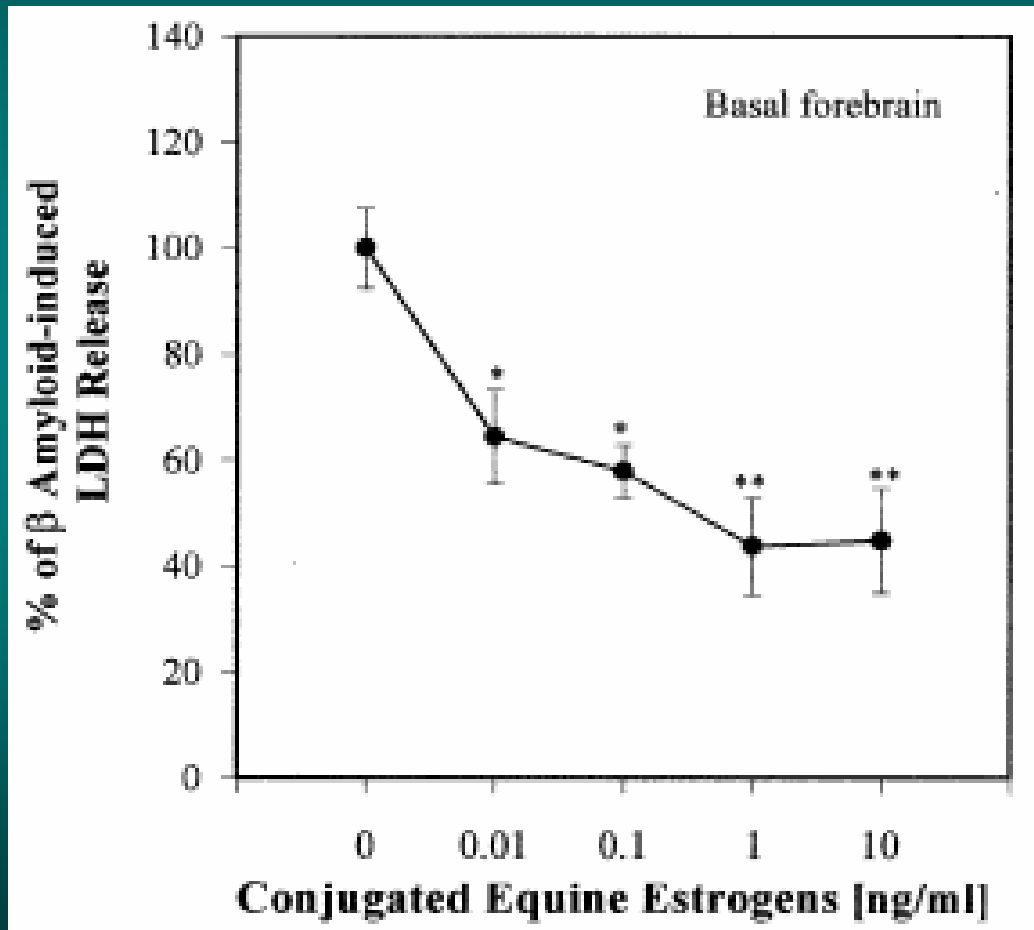
exposure to  $\beta$ -amyloid

24 h

analysis

# Neuroprotective effect of CEE against $\beta$ -amyloid-induced toxicity

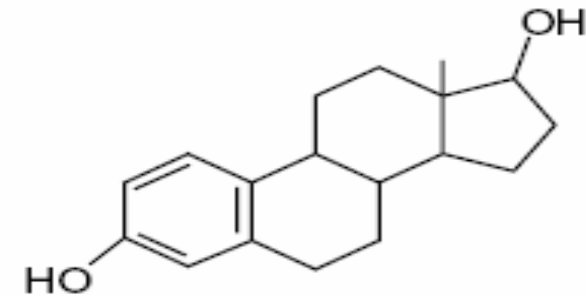
(Hippocampal neurons cultured *in vitro*)



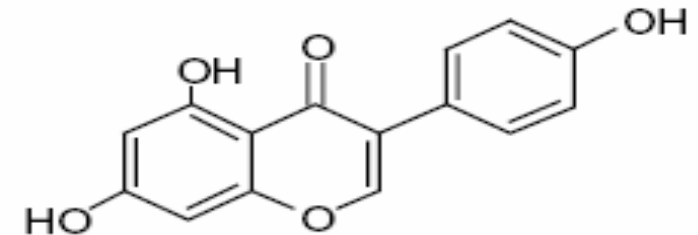
# Phytoestrogens

- Phytoestrogens are non-steroid polyphenols present in many plants, including edible plants.
- Plants produce phytoestrogens e.g. as chemoattractants for *Rhizobium*, as the protective compounds against bacteria and fungi, and as a repellent against plant-eating animals.
- Isoflavonoids (one of four classes of phytoestrogens) activate ER in vitro,
- Their activity is, however, very different ranging usually from 1/1000 to 1/500 of estradiol activity)

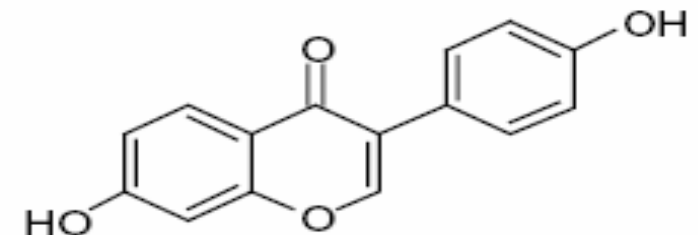
17 $\beta$ -Estradiol



Genistein



Daidzein



# Genistein

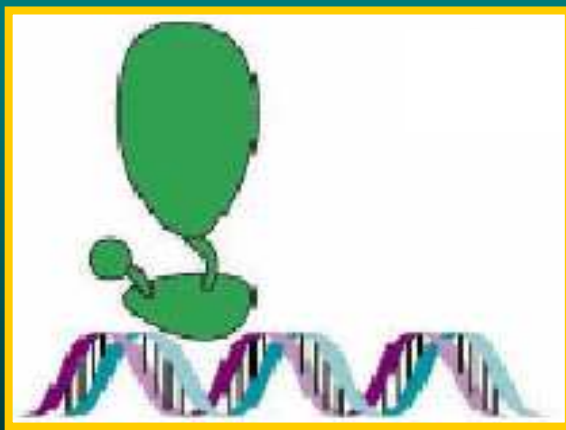
- Partial agonist of ER with 30 x higher affinity to ER $\beta$  than to ER $\alpha$ .
- Reversed correlation between genistein concentration in the blood and frequency of breast cancer, cardiovascular diseases, menopause disorders and osteoporosis were reported.
- Proposed as an alternative of supplementary hormone therapy, as menopause disorders are less pronounced in the countries with a high consumption of soya (hot flushes: 80% in Europe, 18% in China). In China and Japan the bigger problems are headache and backache. In Chinese medicine, to relieve these symptoms, the plants rich in phytoestrogens are recommended.
- Some data suggest that soya-rich diet may decrease the hot flushes, but doses required are very high.
- Clinical trials **did not** confirmed any beneficial effects of soya.

**Genistein is an inhibitor of tyrosine kinases**

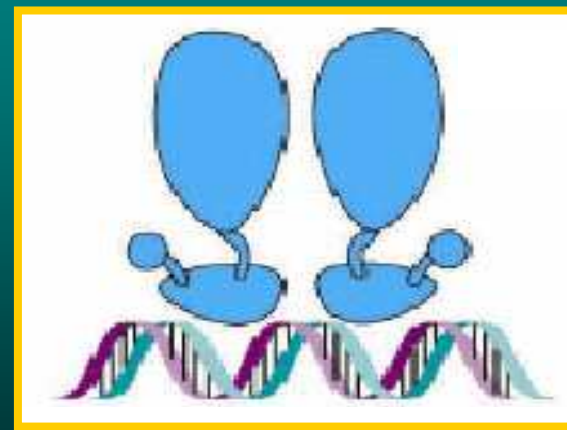


# ERR - estrogen related receptor

- Still **orphans**, but waiting for adoption (perhaps will be adopted soon).
- ERR family, closely related to estrogen receptors (ER), contains three similar members: **ERR $\alpha$** , **ERR $\beta$** , **ERR $\gamma$** .
- ERRs bind to DNA as **monomers** (then recognize the same sequence as SF-1 orphan nuclear receptor, steroidogenic factor-1) or as **homodimers** (then it recognize estrogen receptor response element). In the cytoplasm they are connected to Hsp90.



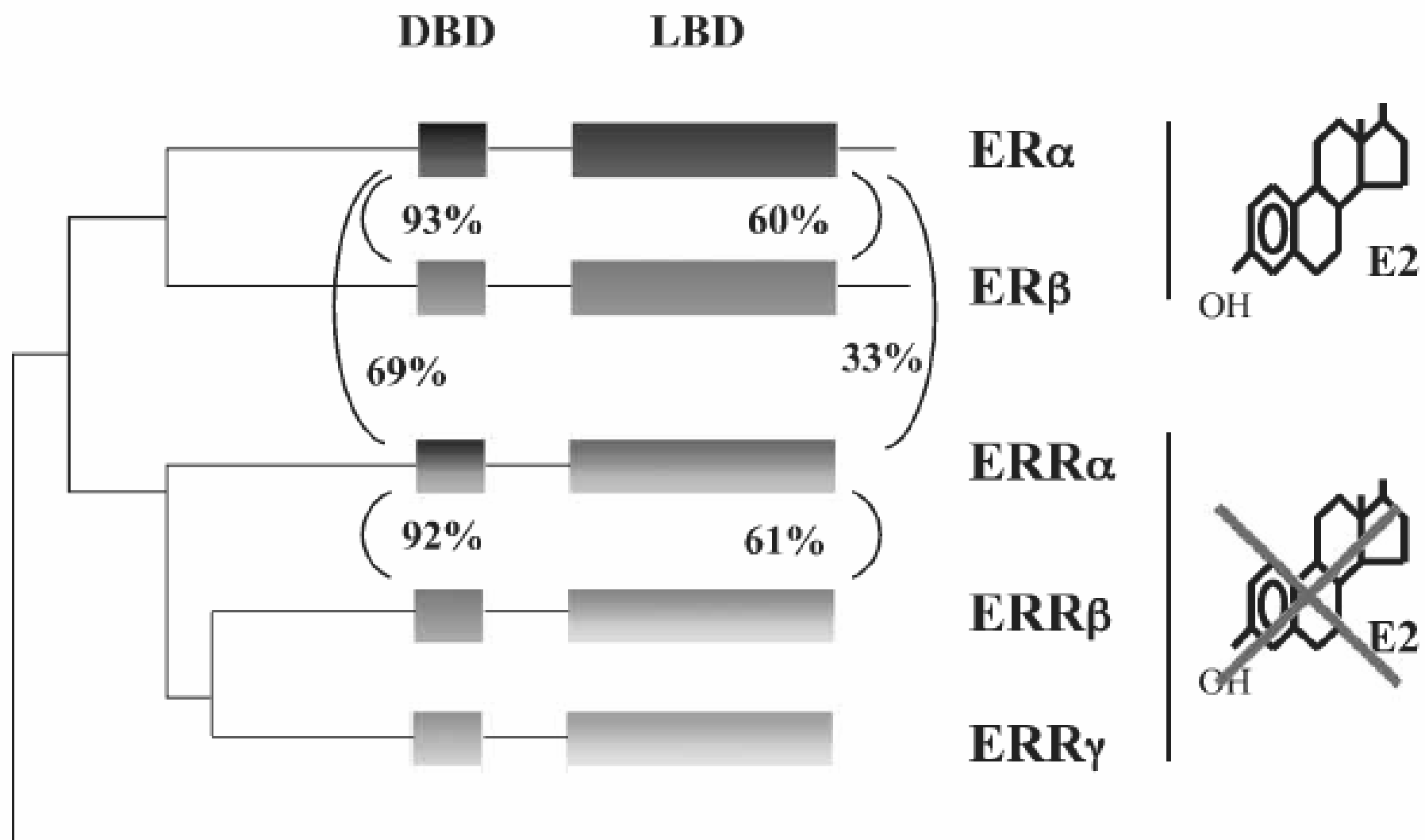
SF-responsive element



ER-responsive element

# ERR - estrogen related receptor

- ERR generally repress transcription of target genes, but they can also activate transcription.
- Perhaps in vivo they can be constitutively active, as even without any ligand they can interact with coactivators.
- ERR cannot bind estrogens in vitro, despite some homology in LBD (35% aminoacid similarity).
- Homodimers may recognize estrogen responsive elements (67% aminoacid similarity in DBD). Therefore they can possibly target all genes regulated by estrogens.
- They were shown to modulate the expression of genes regulated by estrogen receptors in bones and breast tissues.



**Figure 1** The ER/ERR subfamily A schematic representation of the two mouse ERs and the three mouse ERRs is displayed. Percentage of sequence identity within the DBD and the LBD is indicated.

# Expression of ERR

- **ERR $\alpha$**  expression is found in embryo in:

- \* trophoblast,
- \* visceral yolk sac,
- \* primitive heart
- \* **neural tube and then in brain and spinal cord**
- \* bones

- **ERR $\beta$**  in embryo:

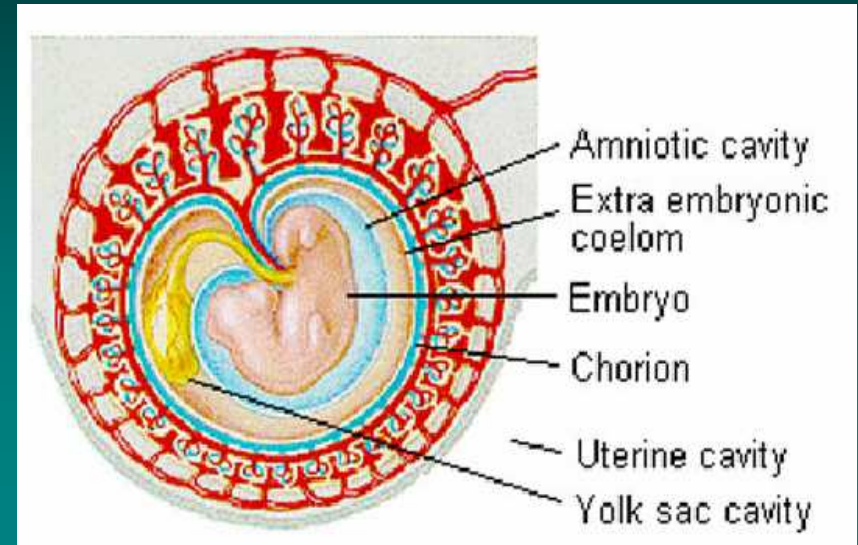
- \* early chorion - play a role in early placenta formation

- **ERR $\gamma$**  in embryo:

- \* **brain** and also kidney, lung, liver

- **ERR $\gamma$**  in adults:

- \* **brain, spinal cords**, and also lung, bone marrow, adrenal and thyroid glands etc.





# Function of ERR

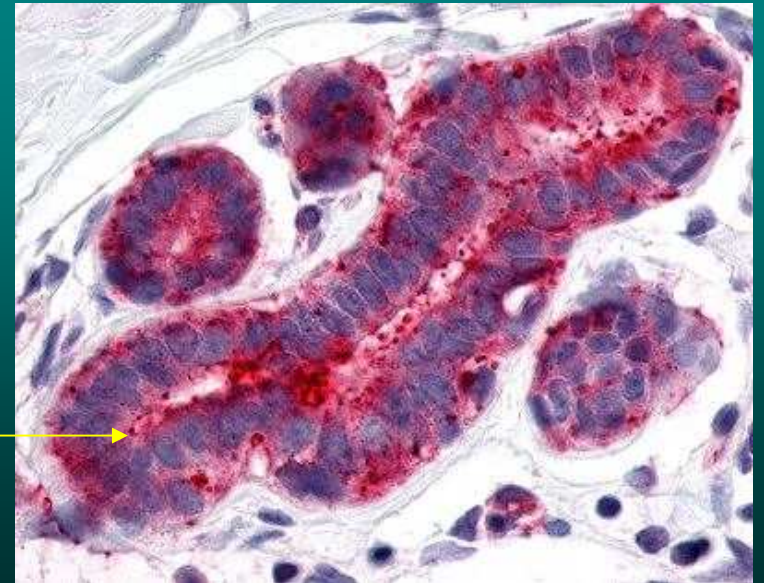


- ERR play a role in **bone** formation:
  - \* They are expressed in ossification zones of the mouse embryo and up-regulates the expression of the osteopontin gene.
  - \* They play a role in *in vitro* osteoprogenitor cell proliferation and differentiation.
- In **adipose tissues**, ERR participate in the control of energy expenditure and fat metabolism through the regulation of the expression of the MCAD (medium chain acyl CoA dehydrogenase) gene.
- In the **central nervous system**, ERR are highly expressed but their role is still unknown.
- ERR is mainly expressed during **early embryonic development** where they are necessary for proper trophoblastic cell proliferation and differentiation.

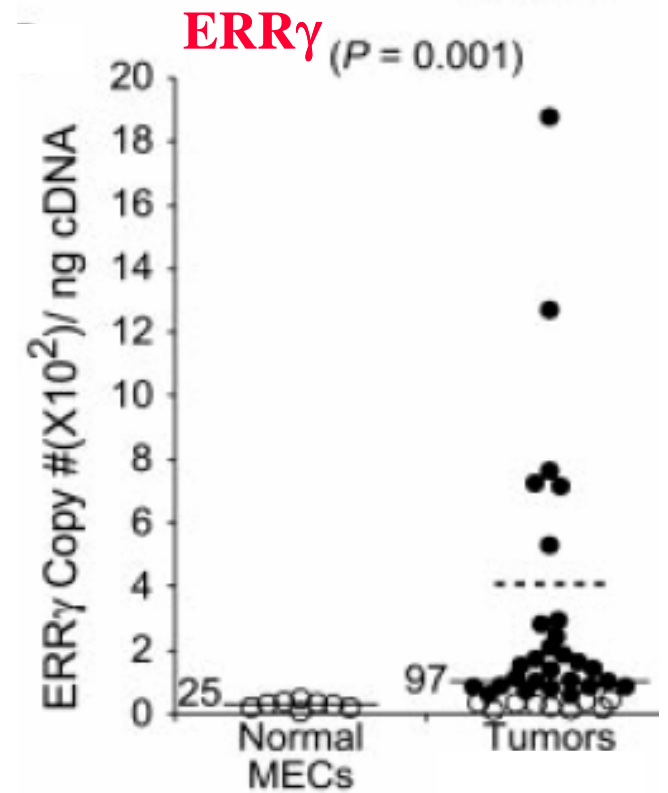
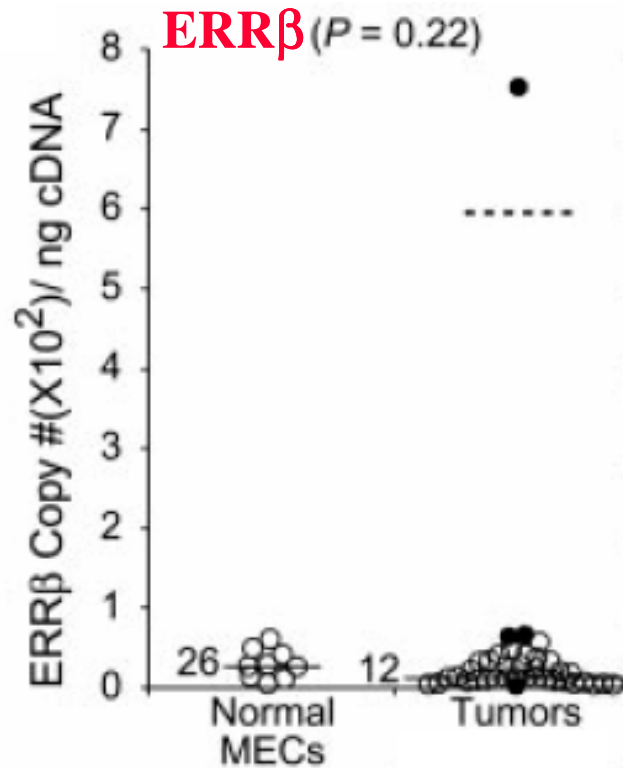
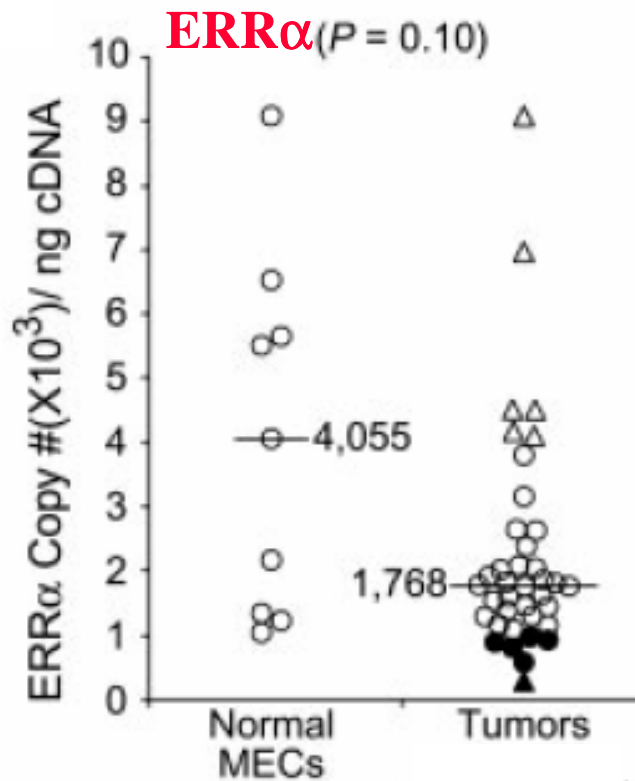
# Ligands of ERR

- No natural ligands are known so far.
- Synthetic ligands:
  - \* Diethylstilbestrol (DES, bind to ERR $\alpha$ , ERR $\beta$ , and ERR $\gamma$ )
  - \* **4-hydroxytamoxifen** (4-OHT, binds to ERR $\beta$  and ERR $\gamma$ ) repress their constitutive transcriptional activity. Raloxifen does not bind to ERR.
- These drug molecules have a long history of use in women's health, through their opposing effects on the activity of the classical ER.
- It has been suggested that ERR regulators may be used for treatment of breast cancer.

breast epithelium  
stained for ERR $\gamma$



# ERR and breast cancer



# ER and ERR ligands

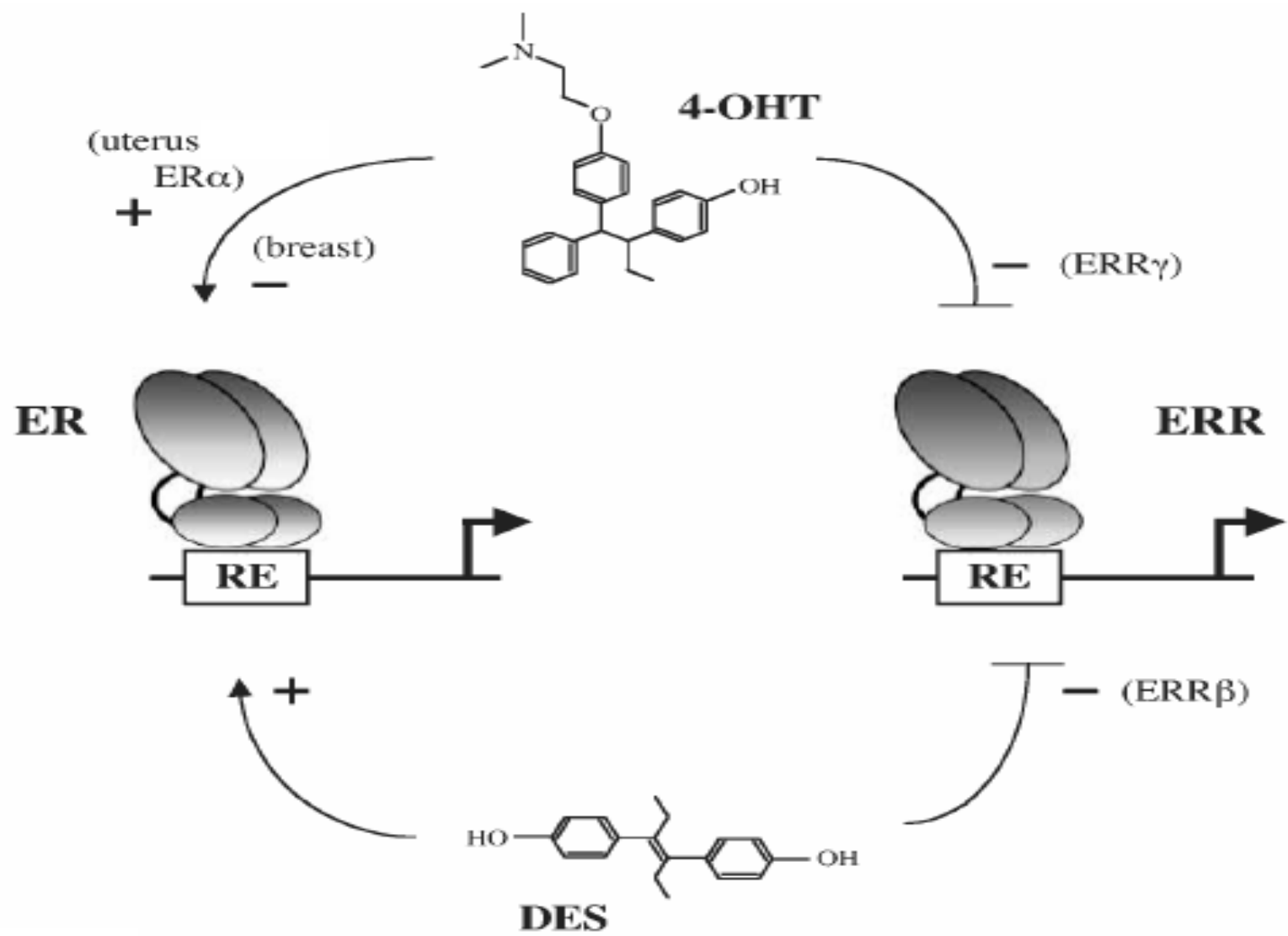
	ESTRADIOL	DES	4-OHT	RALOXIFEN
ER $\alpha$	Agonist	Agonist	Antagonist/Agonist	Antagonist
ER $\beta$	Agonist	Agonist	Antagonist/Agonist	Antagonist
ERR $\alpha$	—	Antagonist	—	—
ERR $\beta$	—	Antagonist	Antagonist	—
ERR $\gamma$	—	Antagonist	Antagonist	—

# Diethylstilbestrol (DES)

- DES, a synthetic estrogen, was widely used between the 1940s and the 1970s to prevent spontaneous abortion in women. However, **patients exposed *in utero* to high doses of DES** have a range of gynecological troubles, including a **higher incidence of vaginal cancers and malformations of the reproductive tract.**
- DES is a potent **estrogen receptor agonist**, it can therefore be intuitively thought to act through ER and mimic the broad spectrum of E2 action. Recent data evidence that DES also **represses the molecular activities of ERRs.**
- However, it should be noted that the DES concentration necessary to achieve a half-maximal inhibition of ERR activities is much higher compared with its ER-activating potential.

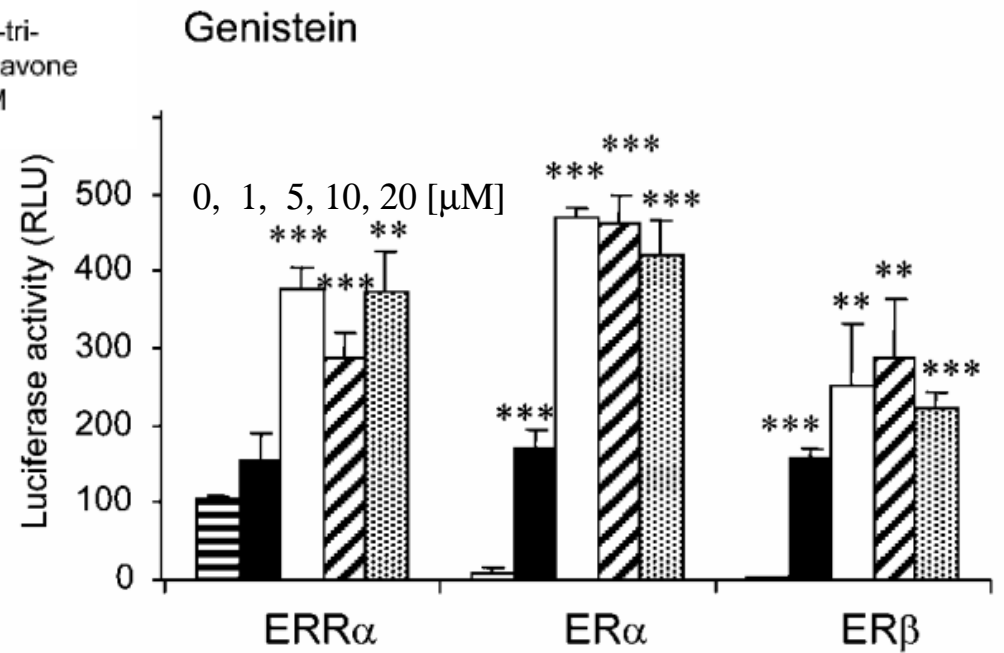
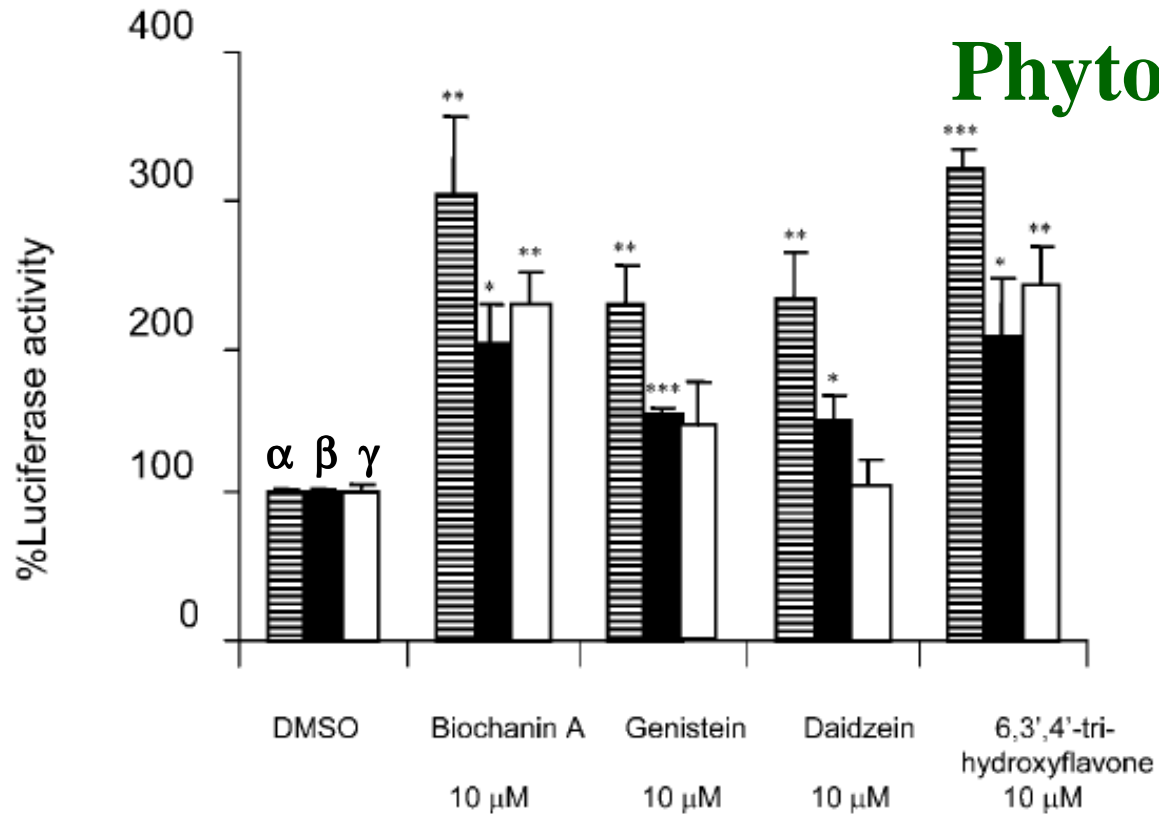
# Tamoxifen (TAM) and 4-OH tamoxifen (4-OHT)

- TAM and 4-OHT are selective ER modulator, and as such displays estrogenic or antiestrogenic actions according to the target tissue. In particular, TAM acts as an estrogen antagonist in the mammary gland and is clinically used for the treatment of breast cancer.
- TAM and 4-OHT disrupt the interactions between ERR and a SRC-1 activator peptide *in vitro*.
- Much higher concentrations (but still pharmacologically relevant) are necessary to obtain inhibition of ERR than ER.
- It could be suggested that some of the antitumor effects of TAM might be mediated by ERR.



Contrasting effects of SERMs on ERs and ERRs. 4-OHT activates ER $\alpha$  in the uterus and bone, but acts as an estrogen antagonist in breast. 4-OHT also deactivates ERR $\gamma$ . DES is a potent ER agonist and inhibits ERR $\beta$ 's transcriptional activities. RE: response element.

# Phytoestrogens and ERs





# ER and ERR interaction

- **ERRs and ERs share common transcriptional target genes, such as lactoferrin, or osteopontin, on which they can either synergize or compete with one another.**
- **Human ERR interact with human ER at least *in vitro* through protein–protein contacts, what possibly increases activity of ER.**
- **The aromatase gene, encoding the enzyme catalyzing the conversion of androgens to estrogens, is a positive transcriptional target of ERR.**
- **Altogether, these findings have raised the possibility that the ERRs might directly and indirectly modulate the estrogenic response.**

## Hormone replacement therapy

### WHI trial

Participants had a 23% increase in cardiovascular disease

*(37 vs. 30 cases per 10,000 persons/years)*  
0.34% v 0.30%

and a 38% increase in strokes

*(29 vs. 21 cases per 10,000 persons/years).*  
0.29% v 0.21%

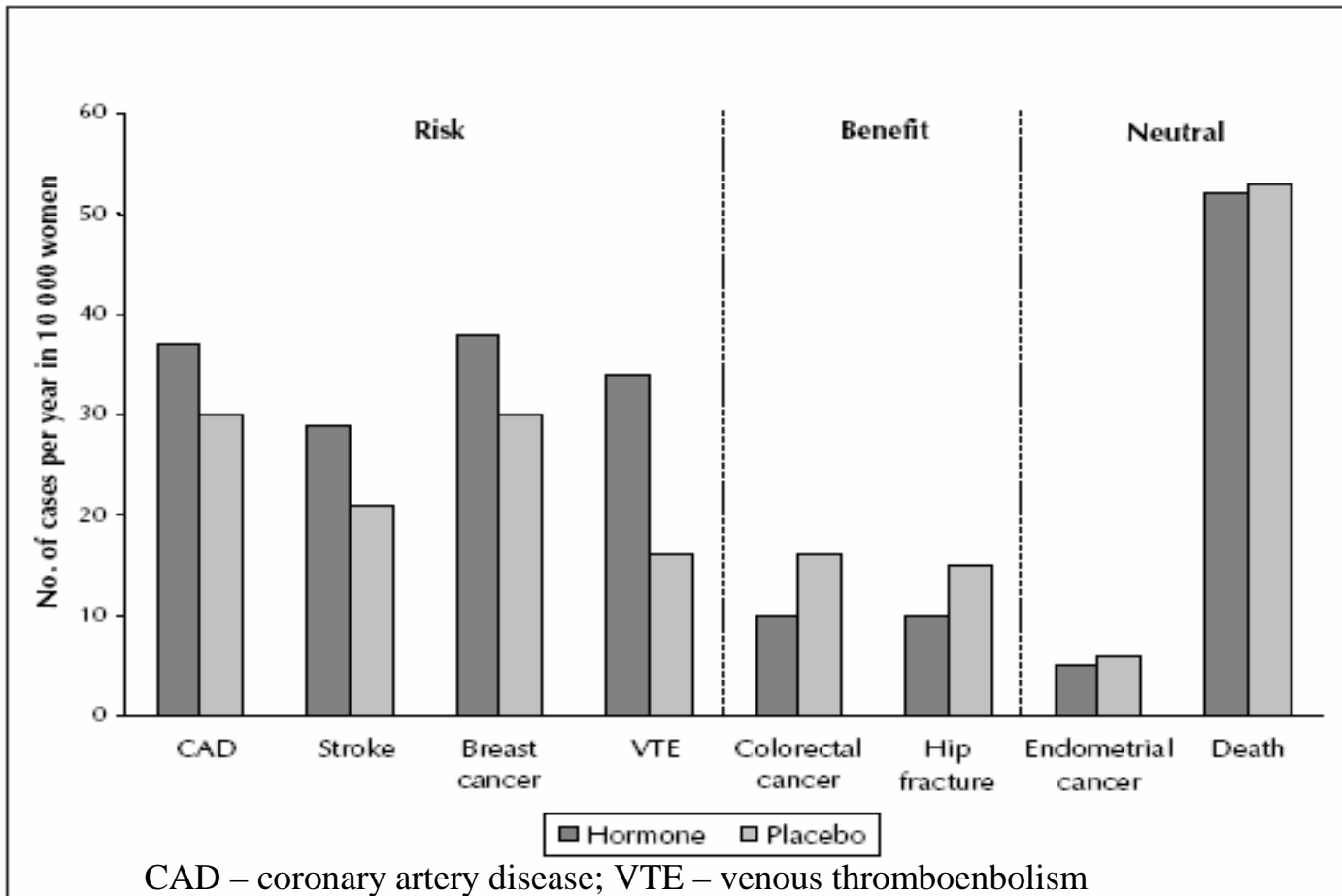


Fig. 3: Effect of HRT on cardiovascular and noncardiovascular outcomes among healthy postmenopausal women in the Women's Health Initiative trial.<sup>14</sup> Outcomes are grouped according to whether HRT had a negative or positive effect, or no effect, compared with placebo. [Source: WHI HRT Update — 2002, National Heart, Lung, and Blood Institute (National Institutes of Health, US Department of Health and Human Services), Bethesda, Md. (available [www.nhlbi.nih.gov/health/women/upd2002.htm](http://www.nhlbi.nih.gov/health/women/upd2002.htm) [accessed 2003 Mar 19]).

Recent data suggest decreased risk of Alzheimer disease (by 67%) and other forms of dementia as well as preservation of cognitive function in women subjected to a long-term HRT (not conclusively confirmed).

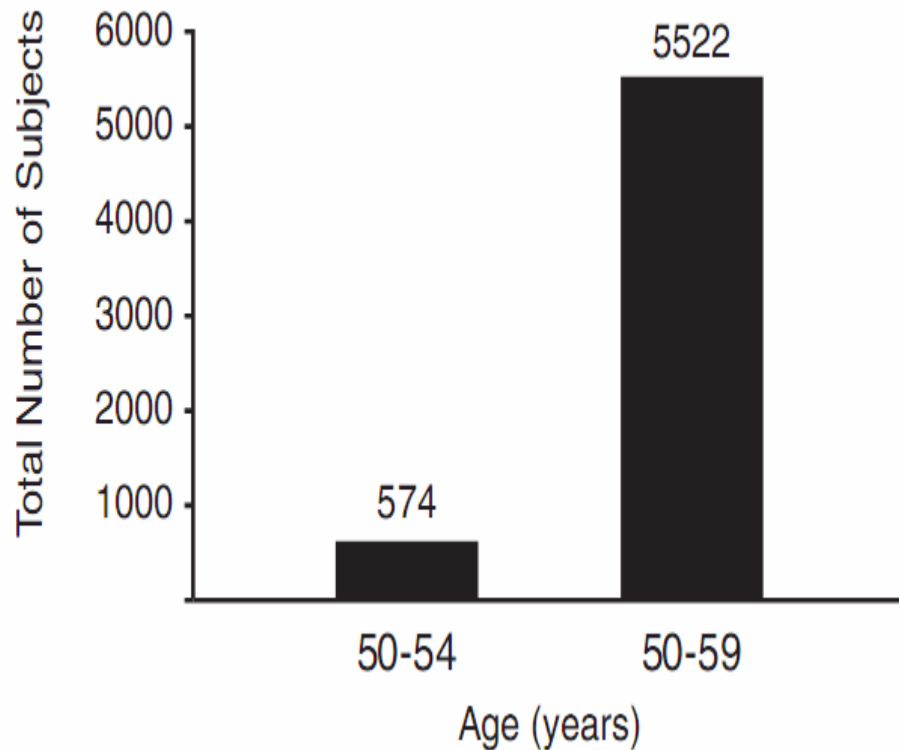
# WHI - not easy for interpretation (?)

The mean **age** in WHI study, it was **63 years** in the E+P subgroup.

The WHI subjects started the hormone therapy at an average of **12 years postmenopause** (in clinical practice: supplementation in the perimenopausal and early postmenopausal periods).

WHI studies combined **a small** (less than 20% of the study population) **healthy group of patients in their early 50s** at the start of the study with a much larger study group of patients in their late 50s to late 70s, many of whom can be assumed to have had advanced subclinical disease.

In the E+P arm of WHI study, only **33%** of hormone-treated and control subjects were **50 to 59** years old, and only **16% to 17%** were **within 5 years of menopause at the time of enrollment**

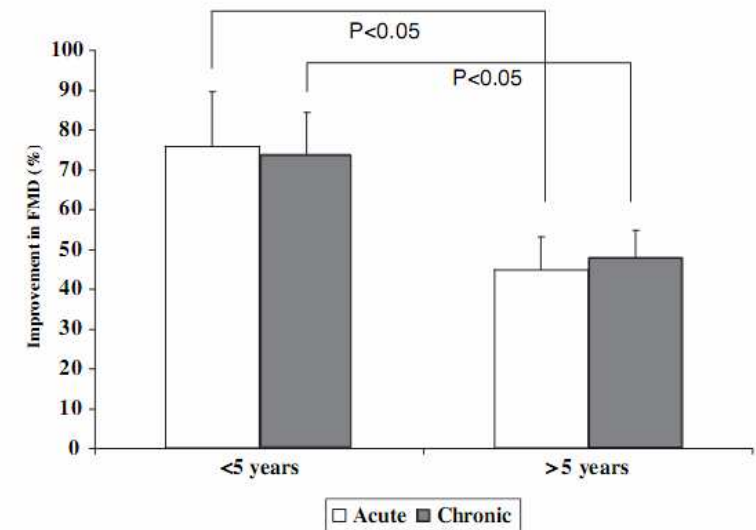


## Number of Events per 10,000 Women per Year of Conjugated Equine Estrogen Therapy Compared to Placebo in the Women's Health Initiative Estrogen Trial by Age at Baseline

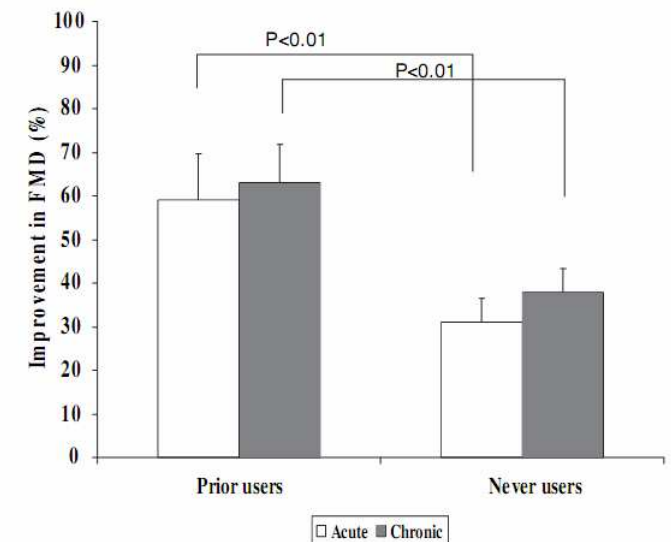


The risks associated with estrogen alone appear to be quite negligible (supported by approximately 40 observational studies that also indicate that initiation of HT early in the postmenopausal period and continued for a prolonged period of time results in a significant reduction of total mortality and CHD).

The window of opportunity for maximal expression of the beneficial effects of HT on CHD appears to be initiation of HT within 6 years of menopause and/or by 60 years of age and continued for 6 years or more.



**Figure 1.** Improvement in FMD after acute and chronic estradiol administration in exogenous estrogen naïve. FMD improved significantly more ( $P<0.05$ ) in women <5 years since menopause than in women >5 years since menopause.



**Figure 2.** Improvement in FMD after acute and chronic estradiol administration in women >5 years since menopause. FMD improved significantly more ( $P<0.01$ ) in those women who had received estrogens in the past than in those who exogenous estrogen naïve.

*Vitale et al. ATVB 2008*

*Nodis HN. Clin Obstet Gynecol 2009.*

# Thank you and see you next week...

## What would be profitable to remember in June:

- Role of ER in neuroprotection
- Ligands for ERR
- ER and ERR interactions

Slides can be found in the library and at the  
**Heme Oxygenase Fan Club page:**

<https://biotka.mol.uj.edu.pl/~hemeoxygenase>

