

Chronic exposure to bisphenol A reduces SULT1A1 activity in the human placental cell line BeWo

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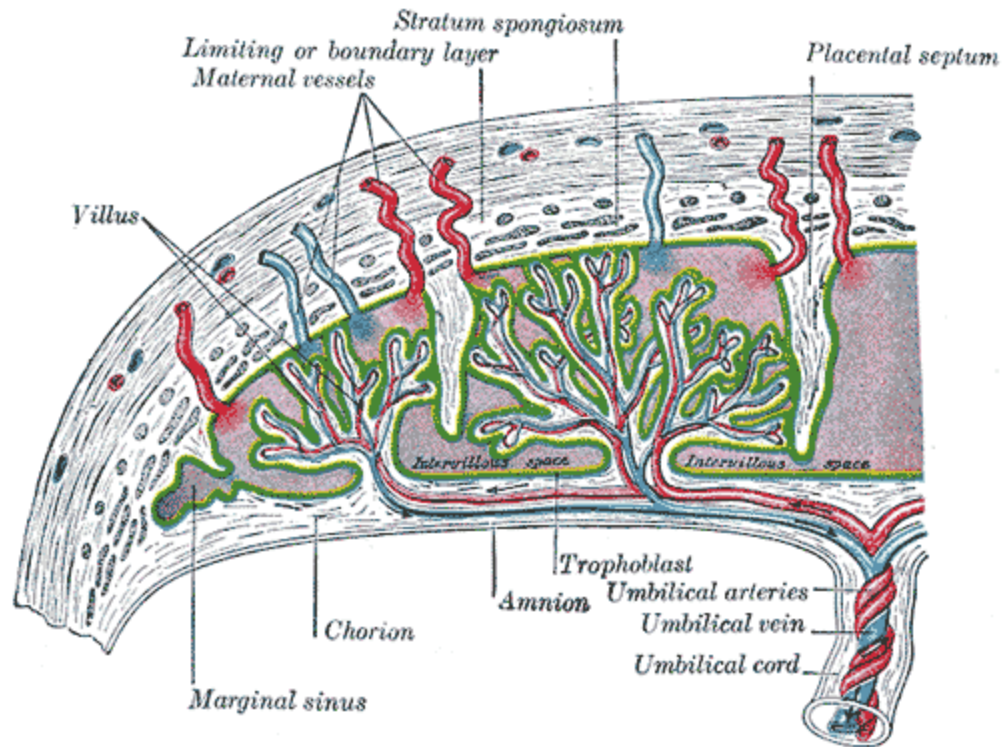
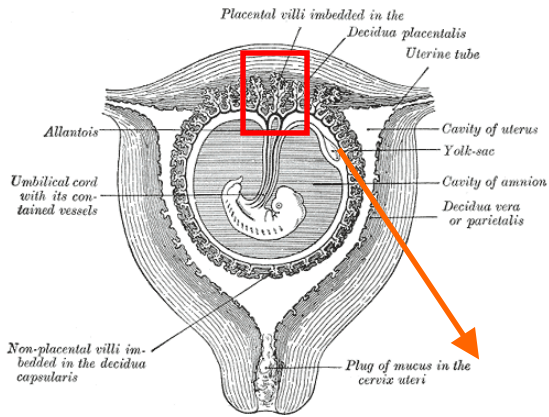
University of Kansas

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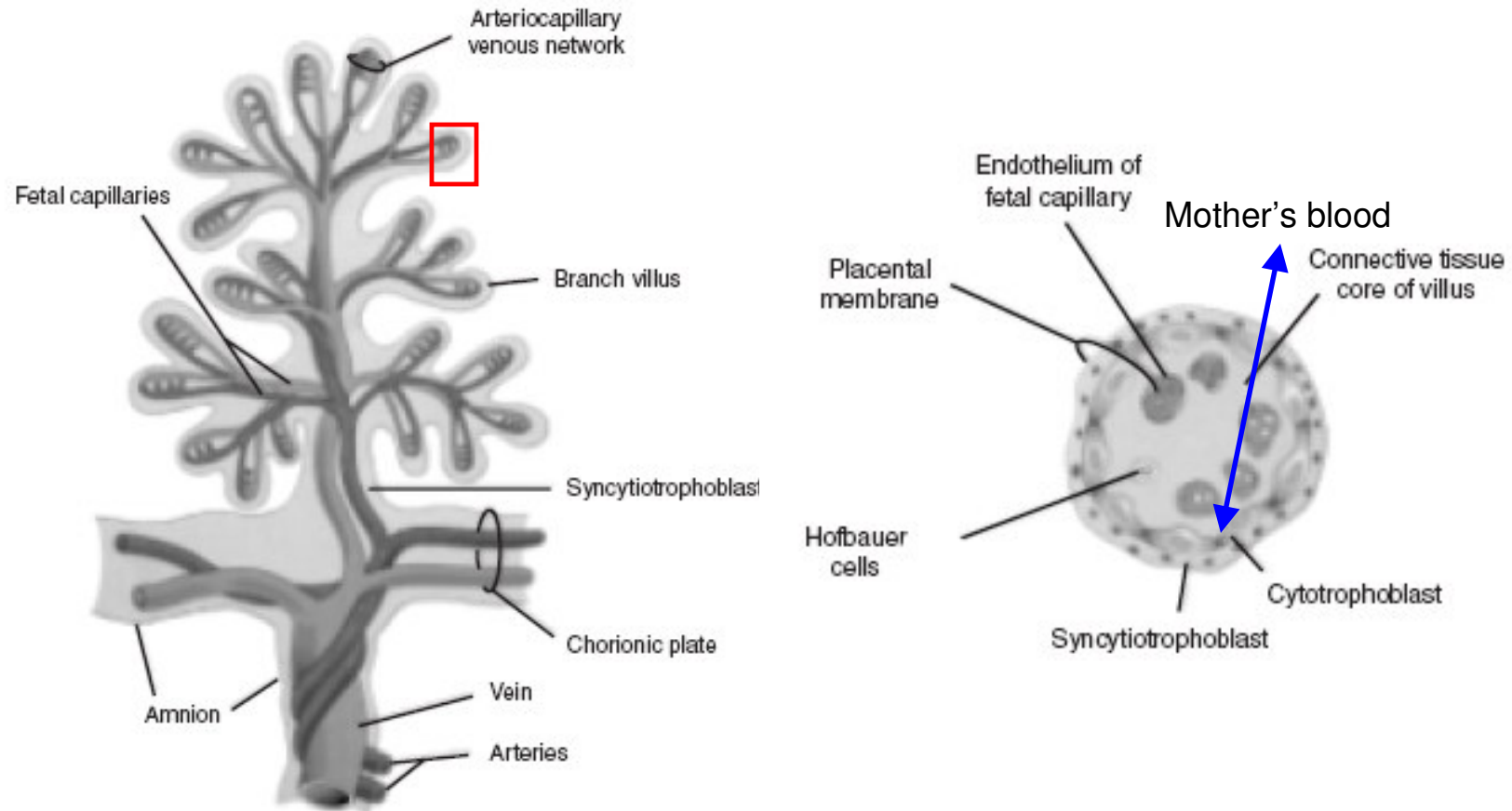
Outline

- Placental structure and models
- Placental permeation
- Placental metabolism and regulation (induction/inhibition)
- Sulfotransferase enzymes in trophoblast
- Bisphenol A
- Effects of bisphenol A on SULT1A1
- Conclusions

The placental barrier



The placental barrier



- Trophoblasts and syncytiotrophoblasts line the maternal villar surface in a monolayer-like fashion.
- Constitute the rate limiting barrier to exchange between the maternal and fetal blood.

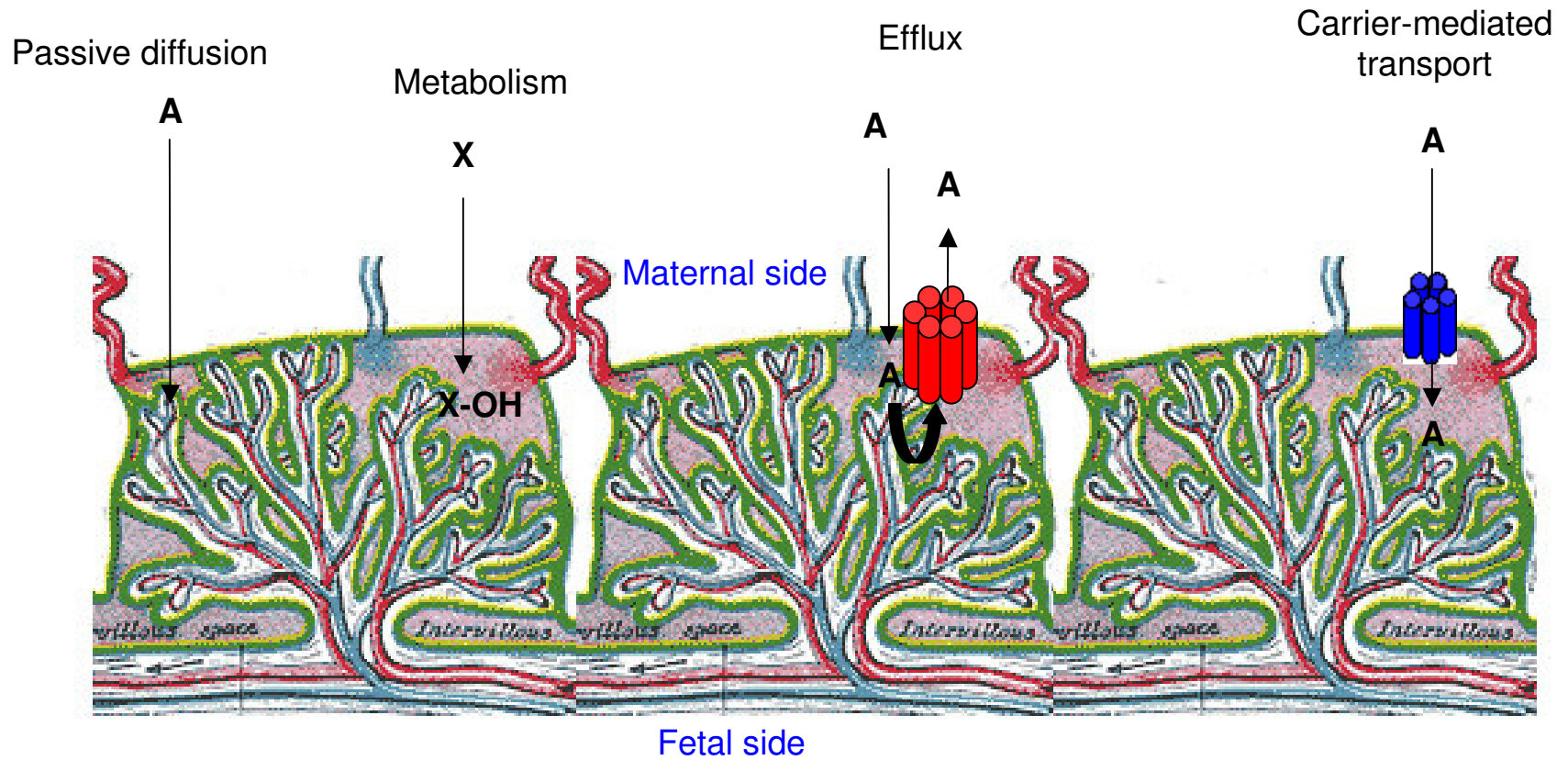
Models of the human placenta

- In vivo models - Anatomical and functional differences between mammalian placentas makes it difficult to extrapolate animal studies to humans.
- In vitro models
 - Perfused placental cotyledon
 - Isolated trophoblast plasma membrane
 - Isolated transporters and receptors
 - Villous explants
 - Primary cultures (cytotrophoblasts)
 - Immortalized cell lines (BeWo, JAr, JEG, HRP-1, etc.)

Refn. Bode et al. In Vitro models for studying trophoblast transcellular transport, *Methods Mol Med.* 2006;122:225-39

Sastry, B.V., *Adv Drug Deliv Rev.*, 1999 Jun 14. 38(1): p. 17-39.

Placental permeation - Factors



Placental metabolism

- Though enzyme expression is much more restricted than hepatic metabolism, those that are functional metabolize xenobiotics as well as hormones.
- Placental enzymes CYP1A1/1A2, CYP19 (aromatase), GST, UGT, SULT
- Maternal blood-borne chemicals (drugs/polychlorinated biphenyls/pesticides) alter expression and activity.
 - Altered steroid metabolism.
 - Altered xenobiotic/drug metabolism.

- Syme et al., Drug transfer and metabolism by the human placenta, Clin Pharmacokinet 2004; 43(8): 487-514
- Avery, M.L., The presence of inducible Cytochrome P450 types 1A1 and 1A2 in the BeWo cell line, Placenta, 2003, 24, 45-52
- Pasanen, The expression and regulation of drug metabolism in the human placenta, ADDR, 38, 1999, 81-97

- Sulfation of drugs (salbutamol, ritodrine, and fenoterol) has been detected in placenta.
- Sulfation is mediated by a family of enzymes - the sulfotransferases.
- Several sulfotransferase isoforms have been detected in term placenta at the mRNA level; some of these are also functionally active.
- Sulfotransferases and UDP glucuronosyltransferases (UGT) act on similar substrates (containing -OH and NH₂ groups).
- Placental UGT activity is low and very variable.

Objective :

1. To characterize the functional activity of selected sulfotransferase isoforms in *in vitro* trophoblast systems (the trophoblast cell line BeWo and primary cytotrophoblasts).
2. Study their regulation (induction/inhibition) by foreign chemicals that accumulate in placenta in significant concentrations.

Sulfotransferases

- Cytosolic - Metabolism of xenobiotics and small endogenous ligands such as steroids, bile acids, and neurotransmitters.

- Membrane-bound - Sulfation of peptides, proteins, lipids; intracellular signaling.

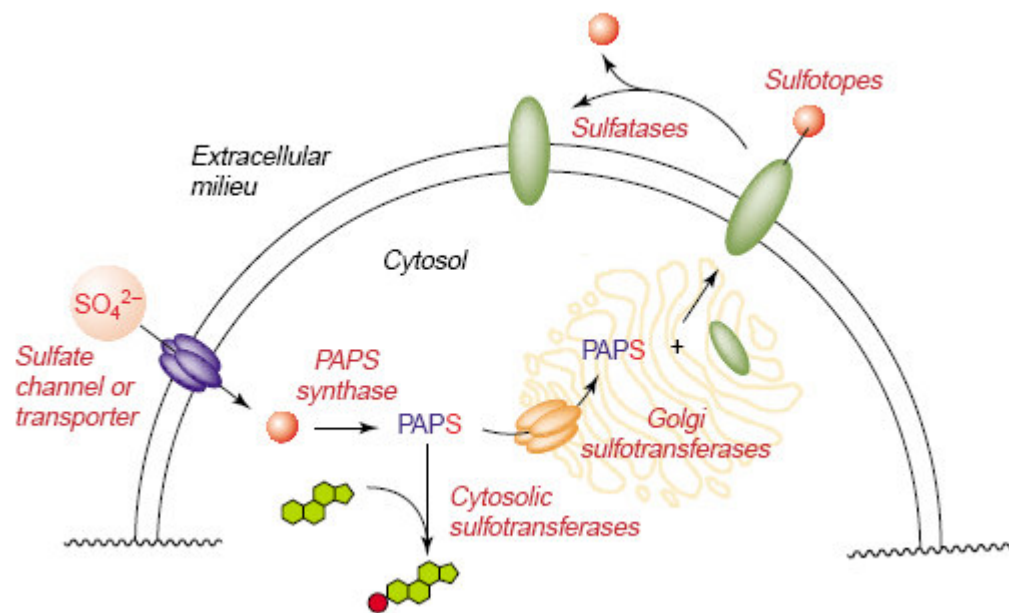
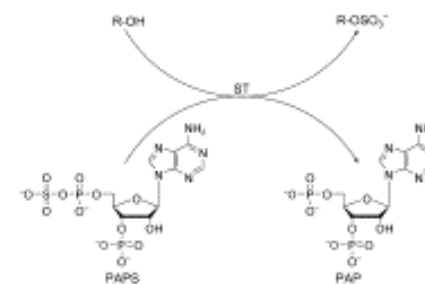
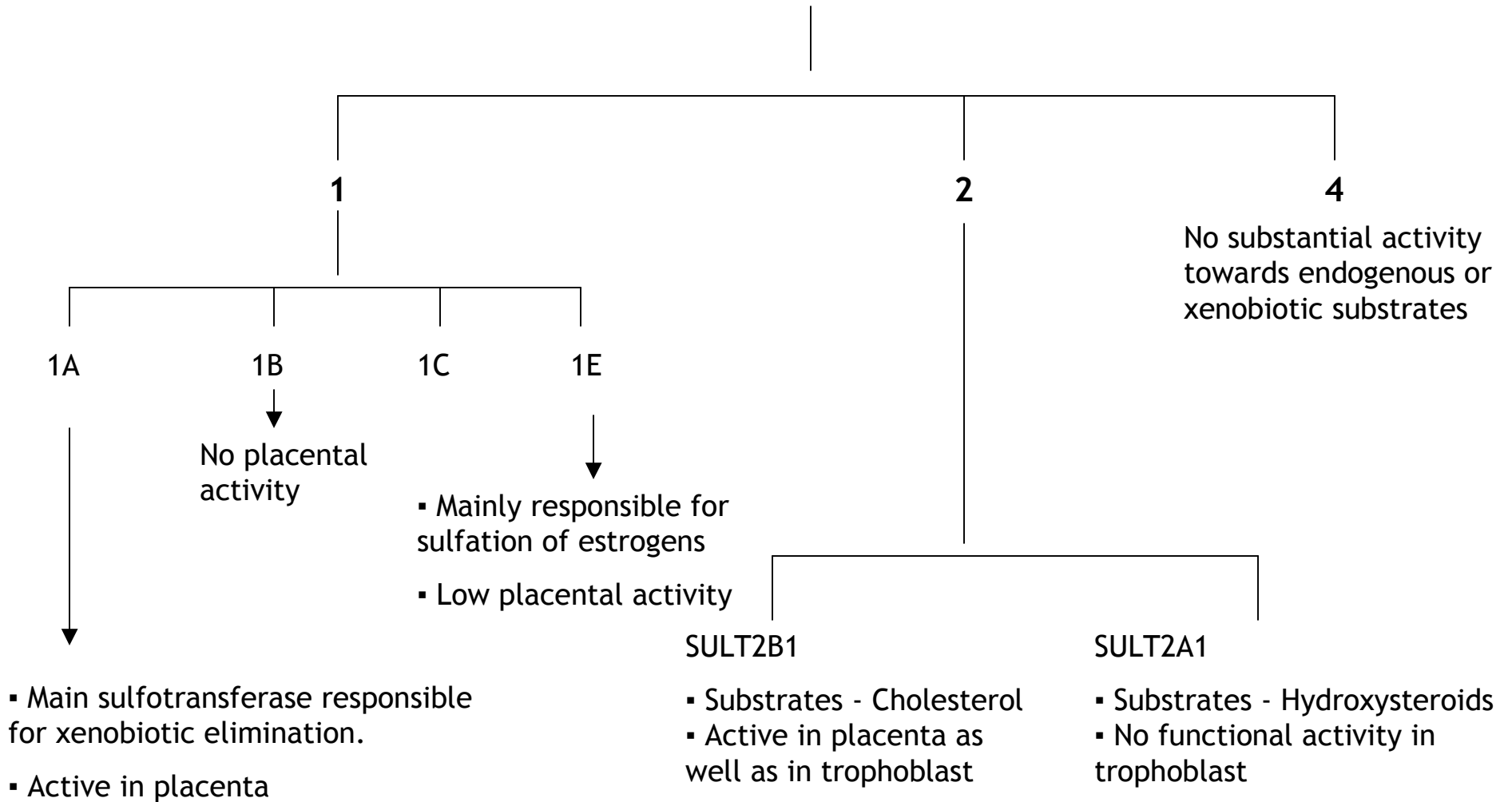


Fig: Hemmerich, S., D. Verdugo, and V.L. Rath, *Strategies for drug discovery by targeting sulfation pathways*. Drug Discov Today., 2004. 9(22): p. 967-75.

Sulfotransferase family in humans



SULT1A1

- Principal human sulfotransferase involved in the elimination of xenobiotics.
- Tissue - Liver, brain, breast, intestine, endometrium, adrenal gland, platelets, placenta, kidney, lung
- Sulfates small phenolic substrates, drugs (minoxidil, troglitazone), hormones such as 17- β -estradiol and thyroid hormones
- Genetic polymorphisms in SULT1A1 (R213H), which cause altered functional activity, have been associated with increased risk of cancer.

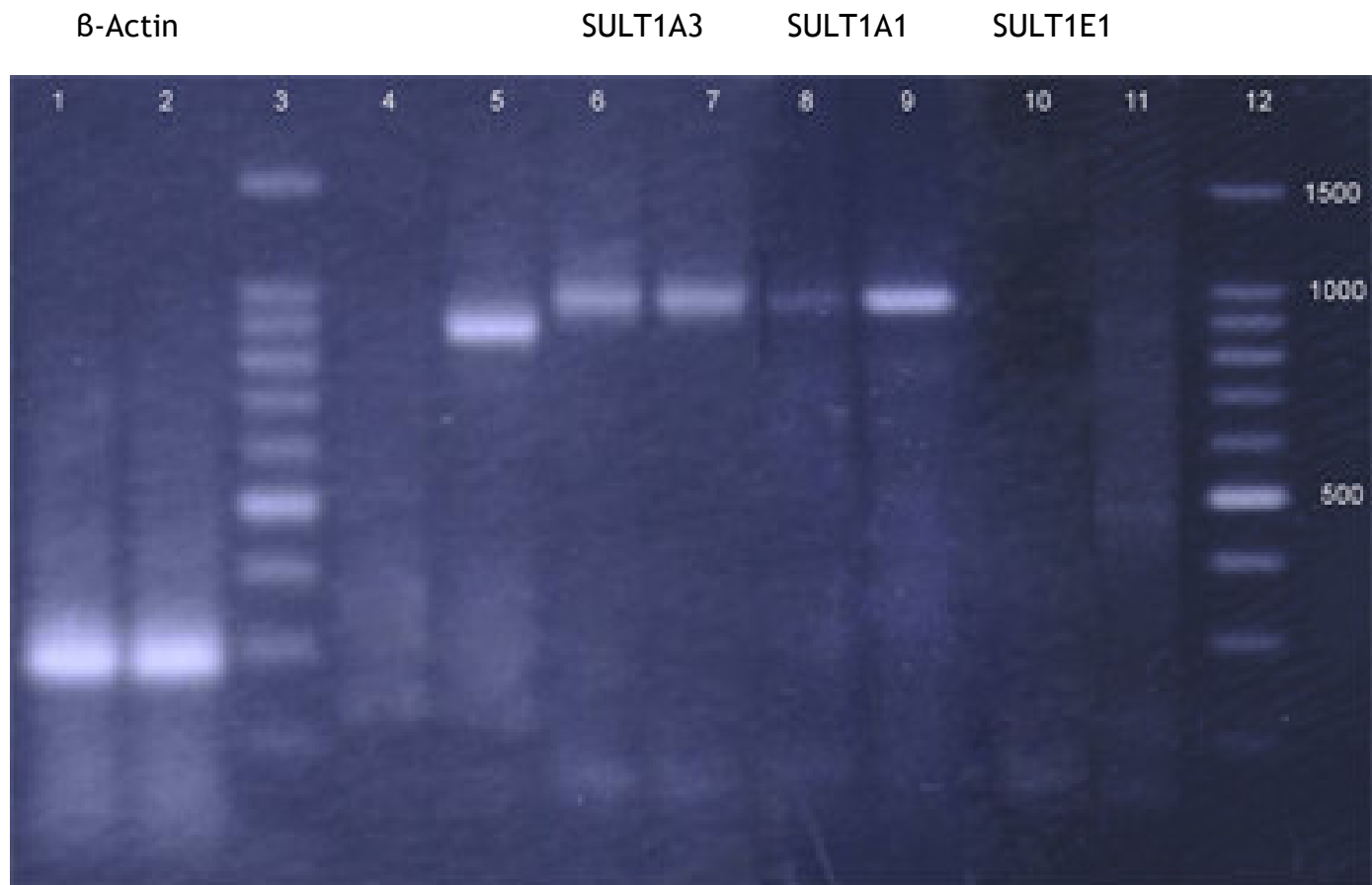
SULT1A3

- Sulfates amines and the high expression level of this enzyme in the intestine has been associated with detoxifying dietary biogenic amines.

SULT1E1

- Tissue - Liver, jejunum, endometrium
- Substrates - Endogenous and synthetic estrogens, iodothyronines
- Deletion of the gene in mice causes placental thrombosis and spontaneous fetal loss.

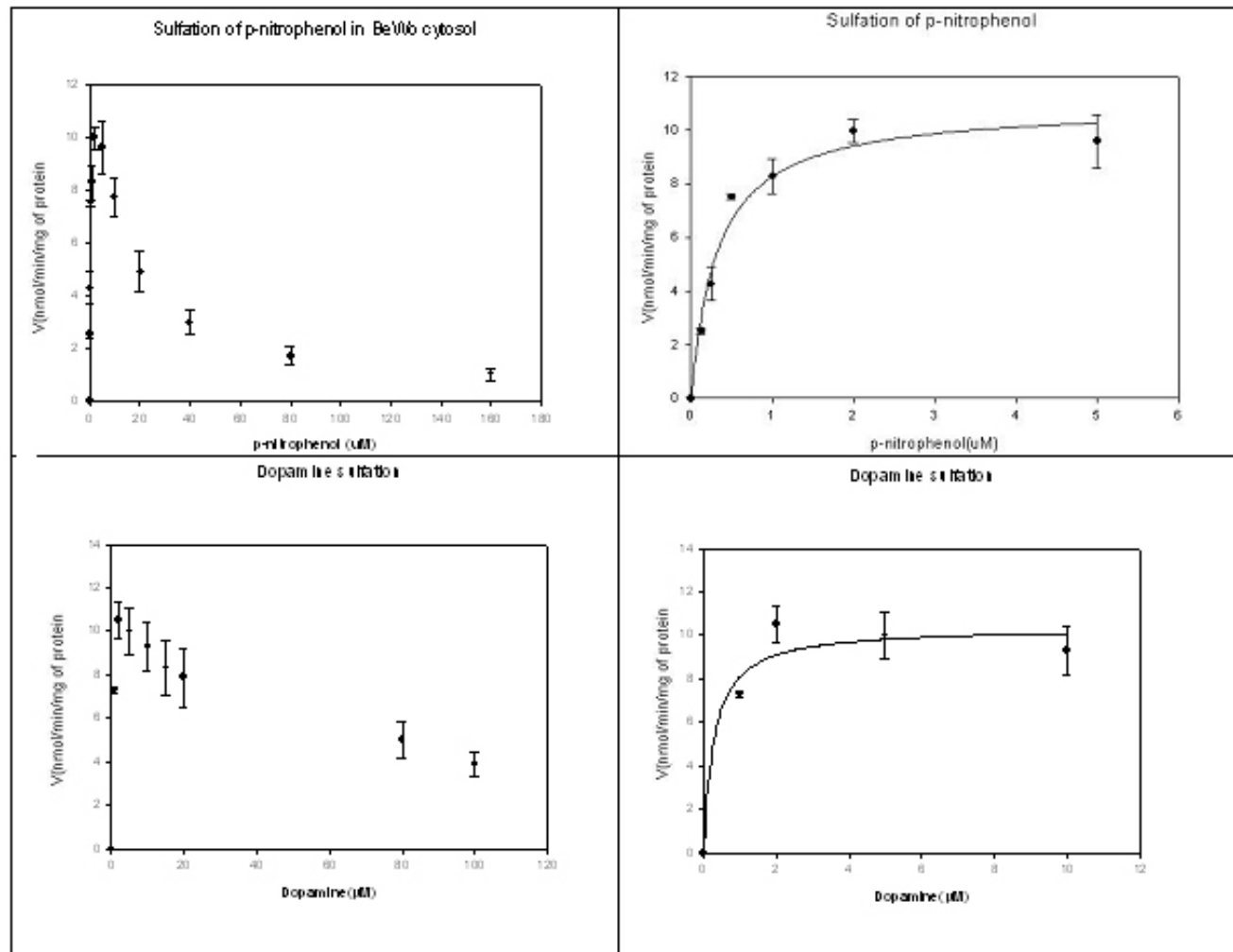
Sulfotransferase mRNA expression in BeWo and in primary trophoblast



Lanes 1, 6,8,10 - Primary cytotrophoblast mRNA probed with primers for β -actin, SULT1A3, SULT1A1, and SULT1E1 respectively

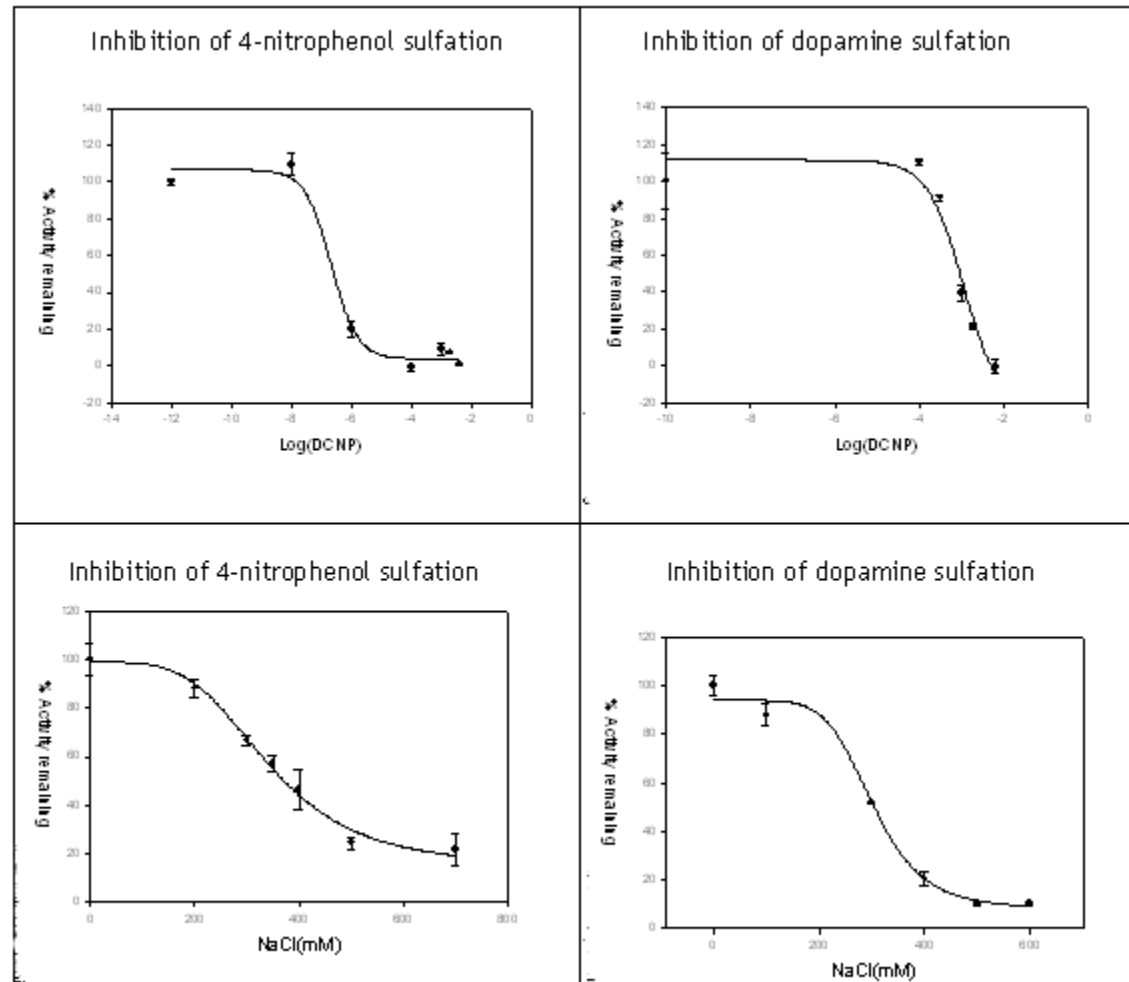
Lanes 2, 7, 9, 11 - BeWo mRNA probed with primers for β -actin, SULT1A3, SULT1A1, and SULT1E1 respectively

Sulfotransferases in BeWo



- 4-nitrophenol - $K_m = 0.33 \pm 0.12 \mu\text{M}$ - Indicative of SULT1A1-mediated sulfation.
- Dopamine - $K_m = 0.5 \pm 0.3 \mu\text{M}$ - Indicative of SULT1A3-mediated sulfation.

Sulfotransferases in BeWo



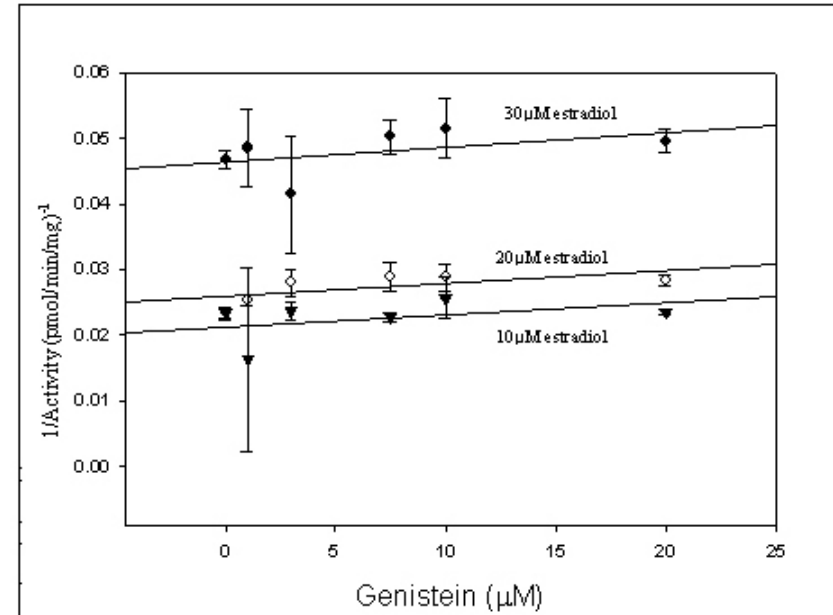
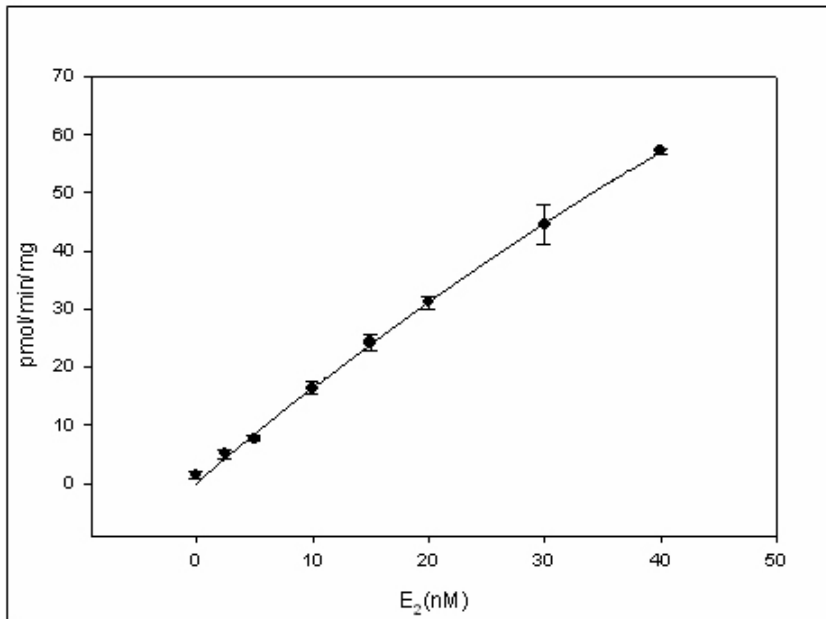
4-nitrophenol sulfation:

$IC_{50, DCNP} = 0.13 \pm 0.1 \mu M$; $IC_{50, NaCl} = 370 \pm 67 mM$; $T_{50} = 42.2 \pm 1.3^\circ C$

Dopamine sulfation:

$IC_{50, DCNP} = 1.07 \pm 0.095 mM$; $IC_{50, NaCl} = 312 \pm 60 mM$; $T_{50} = 39.8 \pm 1.12^\circ C$

Sulfotransferases in BeWo

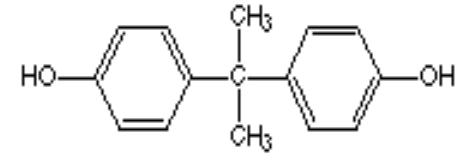


- 17 β -estradiol sulfation - No saturation. SULT1E1-mediated 17- β -estradiol sulfation exhibits saturation in this range.
- Furthermore, genistein a potent inhibitor of SULT1E1 did not inhibit 17 β -estradiol sulfation

Summary of sulfotransferase expression and activity in trophoblast

- In BeWo, both SULT1A1 and SULT1A3 are functionally active but not SULT1E1.
 - * Agrees with sulfation activities reported in term placenta.
 - * BeWo is a good model to study the regulation of placental sulfotransferase enzymes.

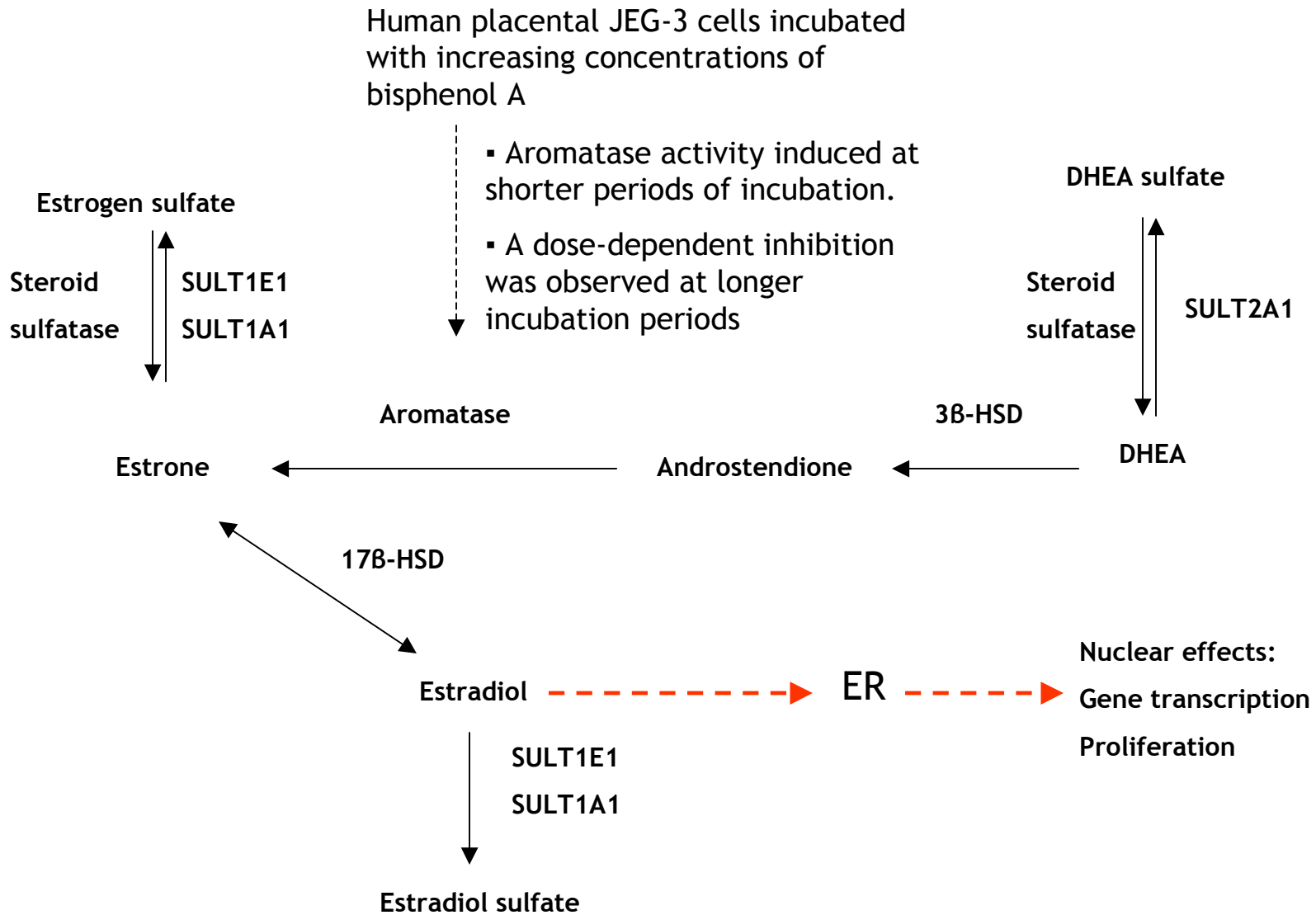
Bisphenol A



- Used in the manufacture of polycarbonate plastics, epoxy resins, dental sealants etc.
- Endocrine disrupting chemical - Mimics the action of natural estrogens and regulates the expression of estrogen-responsive genes.
- Prenatal exposure to BPA -
 - * Up-regulates immune responses.
 - * Causes oxidative stress leading to brain impairment.
 - * Prostate gland enlargement.
- Concentration in amniotic fluid about 5 fold higher than in maternal plasma. Accumulation in placenta is also very high.
- High placental concentrations can alter placental transfer and metabolism.
 - * Alters P-glycoprotein mediated efflux in BeWo

1. Yoshino, S., K. Yamaki, et al. (2004). *Immunology* 112(3): 489-95; 2. Kabuto, H., M. Amakawa, et al. (2004). "*Life Sci* 74(24): 2931-40; 3. Jin, H. and K.L. Audus,. Placenta., 2005. 26(Suppl A): p. S96-S103; 4. Schonfelder, G., W. Wittfoht, et al. (2002). *Environ Health Perspect* 110(11): A703-7.

What about the effects of bisphenol A on metabolism?



How do estrogenic compounds affect SULT1A1 and SULT1A3?

- SULT1A1

- Sulfates several endogenous and exogenous estrogens including estradiol.
- Acutely inhibited by estrogenic compounds.

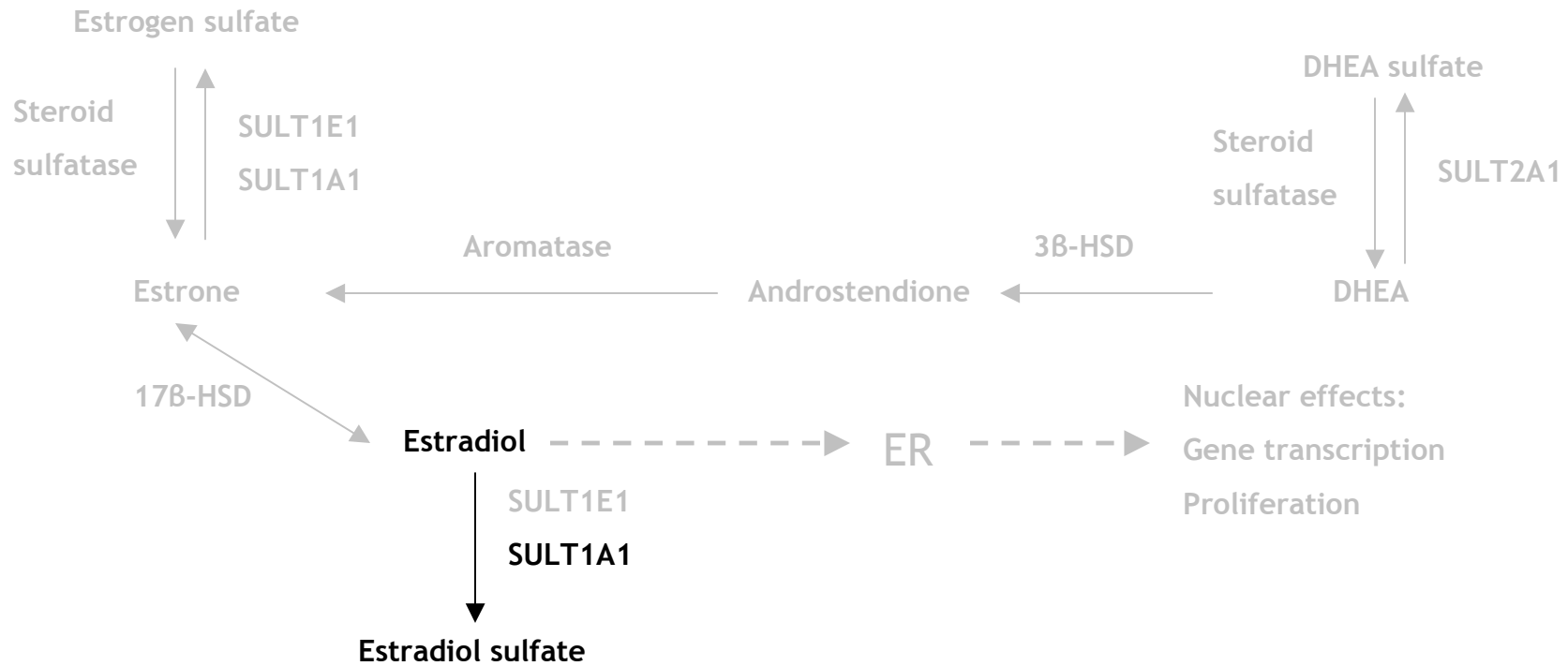
- SULT1A3

- Phytoestrogens also acutely inhibit SULT1A3 but with a much lesser potency.

Effects of chronic exposure to estrogenic compounds.

-mRNA levels of SULT1A up-regulated by 4-OH-tamoxifen (16 hrs) and by estradiol (72 hrs)*.

*Seth, P., et al., *Phenol sulfotransferases: hormonal regulation, polymorphism, and age of onset of breast cancer*. Cancer Res., 2000. **60**(24): p. 6859-63.

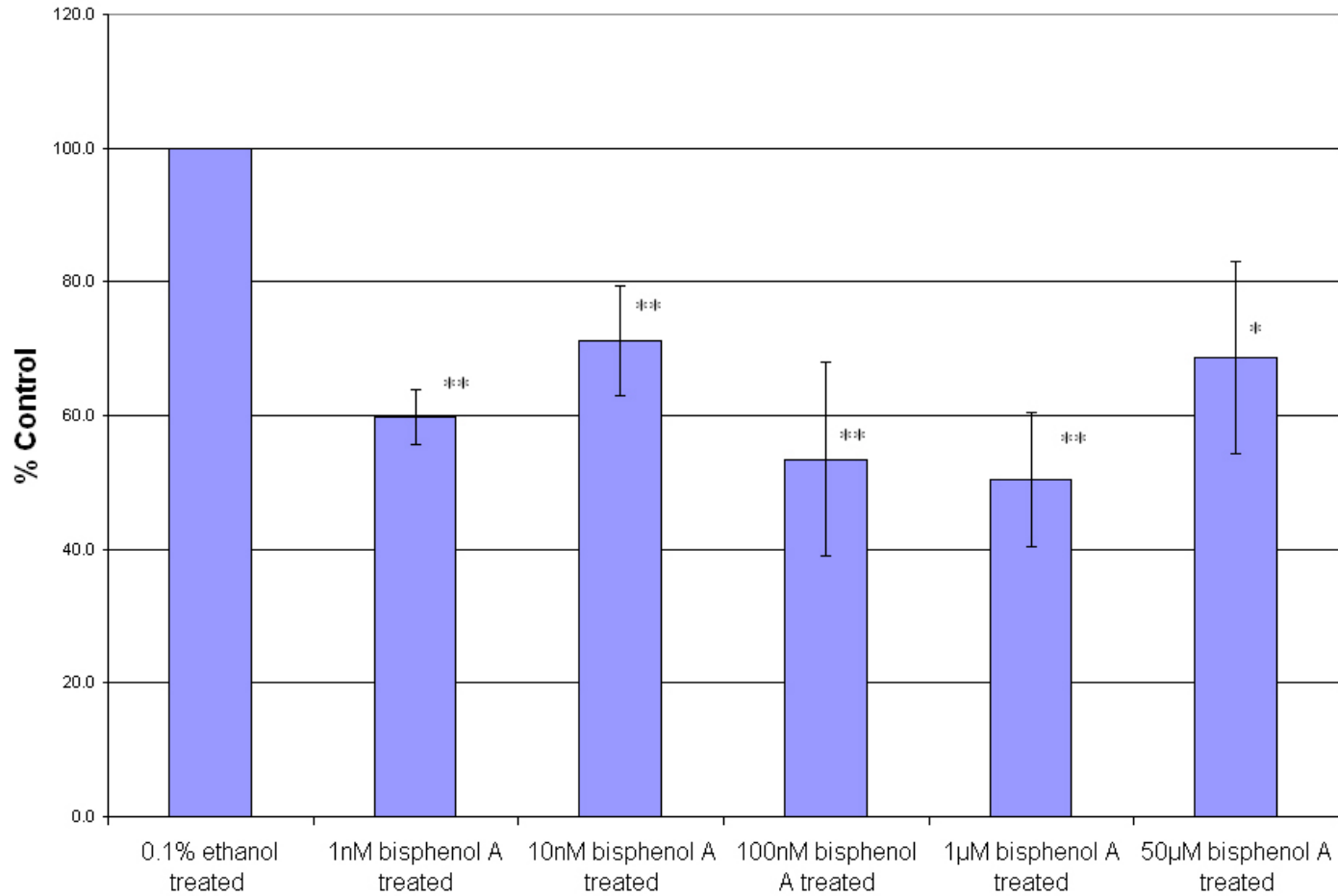


- SULT1A1 is regulated by estrogenic compounds.
- Bisphenol A has high placental concentrations and is estrogenic.
- Bisphenol A alters the metabolic activity of other steroid metabolizing enzymes.
- Does it do the same for SULT1A1?

Bisphenol A sulfation in trophoblast

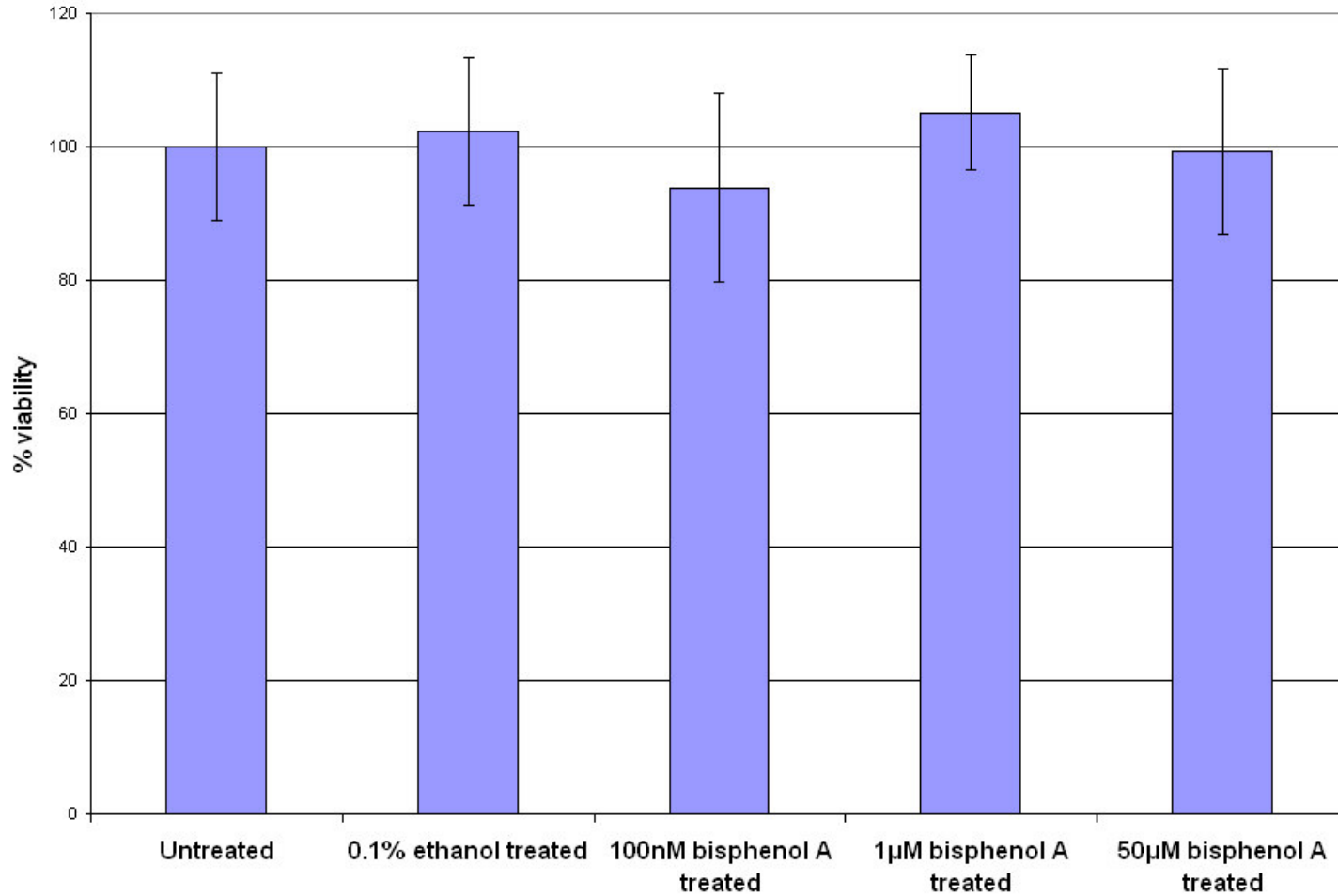
- In vitro, bisphenol A has been shown to be a substrate for both SULT1A1 and SULT1E1 at a concentration of 50 μ M.
- In trophoblast it exhibits negligible sulfation at concentrations ranging from 50nM - 500 μ M.

Effect of chronic exposure (48 hrs) of bisphenol A on SULT1A1 activity



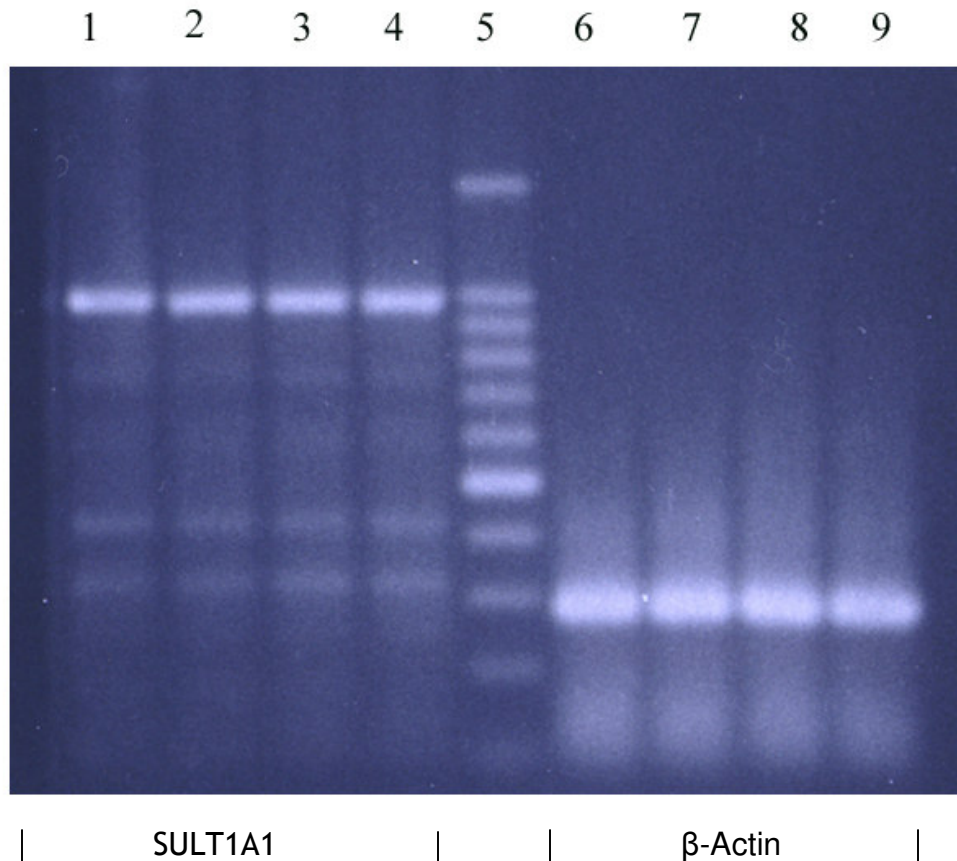
- Significant decrease in SULT1A1 activity at all tested concentrations of bisphenol A

Is bisphenol A toxic to trophoblast cells under these conditions?



There was no significant difference in viability at any of the tested concentrations of bisphenol A

Effect of chronic exposure (48 hrs) of bisphenol A on SULT1A1 mRNA expression



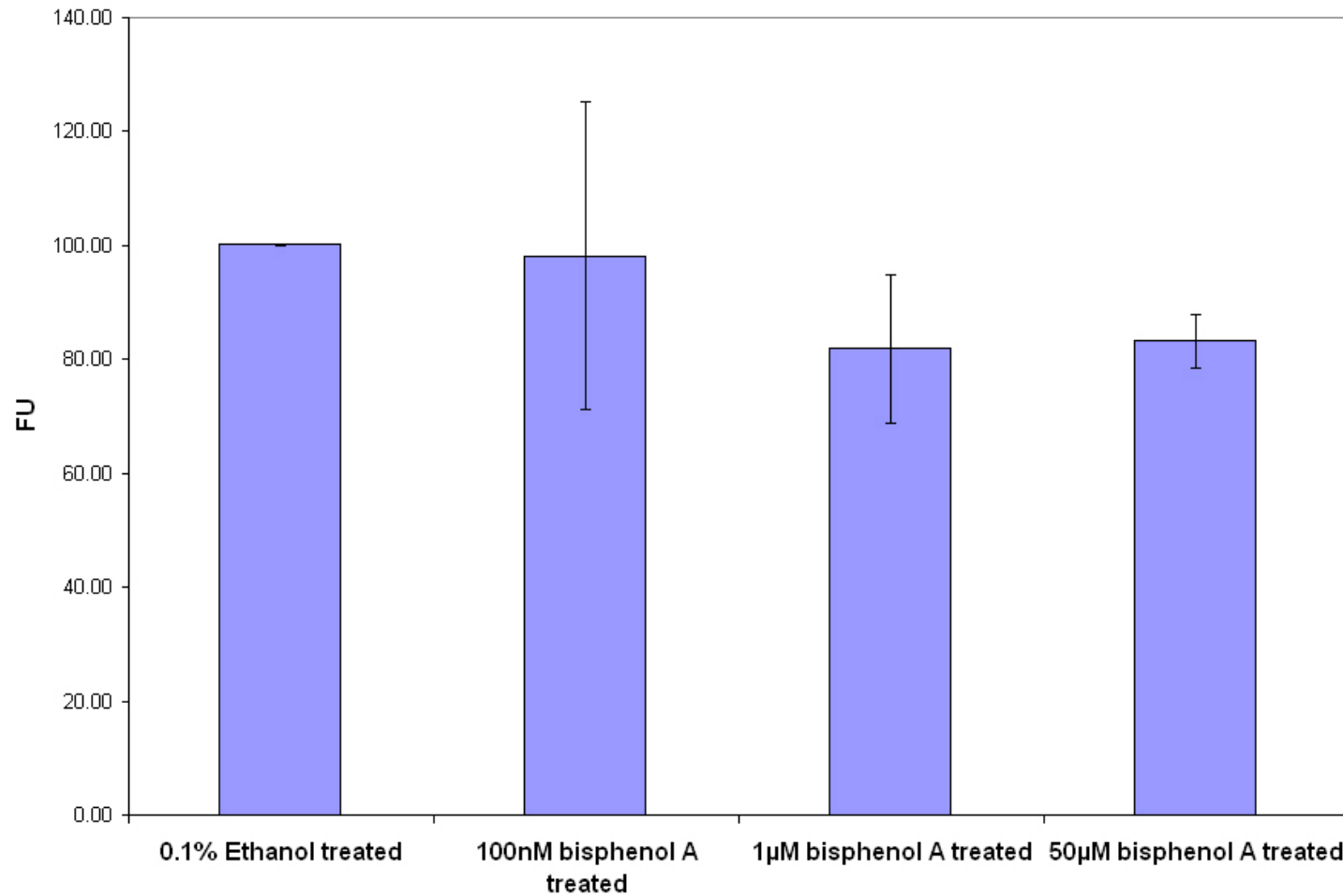
Lanes 1 and 6 - Solvent treated

Lanes 2 and 7 - 100nM bisphenol A treated

Lanes 3 and 8 - 1µM bisphenol A treated

Lanes 4 and 9 - 50µM bisphenol A treated

Effect of chronic exposure (48 hrs) of bisphenol A on SULT1A1 mRNA expression



Bisphenol A treatments did not produce any significant difference in SULT1A1 mRNA expression

Conclusions

- The phenolic sulfotransferases SULT1A1 and SULT1A3 are functional in trophoblast tissue.
- The endocrine disrupting chemical bisphenol A significantly decreased SULT1A1 activity at all tested concentrations (100nM-50µM).
- This effect was not observed on mRNA expression suggestive of post-translational regulation.

Future work

- Bisphenol A as an acute inhibitor of SULT1A1
- Other estrogenic substances as regulators of placental sulfotransferase enzymes.

Acknowledgements

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