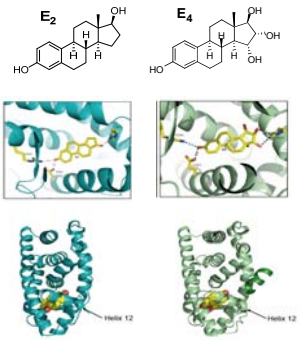
The uterine and vascular actions of Estetrol delineate a distinctive profile of Estrogen Receptor α modulation, uncoupling nuclear and membrane activation.

Coralie Fontaine[*1], Anne Abot[*1], Mélissa Buscato[1], Romain Solinhac[1], Anne Drougard[1], Aurélie Fabre[1], Shyamala Rajan[2], Muriel Laine[2], , Alain Milon[3], Isabelle Muller[3], Daniel Henrion[4], Marine Adlanmerini[1], Christel Péqueux[5] Mélanie Mestdagt[5], Isabelle Raymond-Letron[6], Claude Knauf[1], Pierre Gourdy[1], Gilles Flouriot[7], Philippe Valet[1], Benita S. Katzenellenbogen[8], John A. Katzenellenbogen[8], Françoise Lenfant[1], Geoffrey L. Greene[2], Jean-Michel Foidart[5] and Jean-François Arnal[1]

[1]INSERM U1048, Institut des Maladies Métaboliques et Cardiovasculaires, Université de Toulouse - UPS, Toulouse, France , [2] Department for Cancer Research, University of Chicago, Chicago, Illinois, United States of America, [3] CNRS and Université de Toulouse, IPBS, Toulouse, France, [4] INSERM U1083, CNRS UMR 6214, Université d'Angers, Angers, France , [5] Université de Liège, Groupe Interdisciplinaire de Génoprotéomique Appliquée, Liège, Belgique , [6] Université de Toulouse, INP, ENVT, Toulouse, France , [7] Université de Rennes I, Institut de Recherche en Santé Environnement et Travail, IRSET, INSERM U1085, Team TREC, Biosit, Rennes, France, [8] Departments of Molecular and Integrative Biology and Chemistry, University of Illinois at Urbana-Champaign, Urbana, Illinois, United States of America

Background: Beside the well characterized 17b-estradiol (E_2) that is considered as the active estrogen during the estrous cycle, estriol (E_3) and also estetrol (E_4) are synthesized during pregnancy, but their physiological roles are essentially unknown. E_4 appears to be exclusively produced by the human fetal liver (Hagen et al, 1965). E_4 also differs from E_2 by having a long plasma half-life (about 28 hours) (Visser & Coelingh Bennink, 2009), and it neither stimulates the production of nor binds to sex hormone binding globulin (SHBG) (Hammond et al, 2008). Because of these characteristics, E_4 was evaluated, in combination with a progestin, as a new oral contraceptive in a phase II clinical trial (manuscript in preparation). Very interestingly, E_4 (up to 20 mg/day) did not elicit changes in circulating hepatic factors, and thus might not increase thrombo-embolic events, which are undesirable effects of estrogen pharmaceuticals containing E_2 or ethinyl-estradiol (EE) (manuscript in preparation). Unfortunately, as previously reported (Valera et al, 2012), the impact of estrogen on hepatic factors is species dependent, which precludes the use of mice as an animal model to elucidate these mechanisms in humans. The physiological responses to estrogenic compounds are initiated by their binding to the estrogen receptors (ER), ERa and ERb. E_4 binds ERα with a modest preference over ERβ (Visser et al, 2008). ER mediates its transcriptional activity after ligand binding inducing an ordered sequence of interactions between two activation functions (AF), AF-1 and AF-2,and co-activators such as the steroid receptor coactivator (SRC) 3, a member of the p160 subfamily (McKenna & O'Malley, 2001 ; Metivier et al, 2003; Smith & O'Malley, 2004). In addition, estrogens can act through a distinctly different pathway by inducing rapid extra-nuclear activity *via* the activation of a pool of ERs localized at the plasma membrane, a process termed membrane-initiated steroid signaling (MISS) (Wu et al, 2011) (Ascenzi et al, 2006). Although ERα MISS effects were initially also called "non-genomic" effects, they can modulate ERa dependant transcriptional activity in cultured cell models *in vitro* (La Rosa et al, 2012). However, thanks to a unique mouse model targeted for the ERα palmitoylation site membrane, we recently demonstrated a very contrasted involvement of MISS mediated E2 action in two tissues: the uterus in which the E2 response depends on ERa nuclear action and the arteries involving exclusively MISS of ERa to mediate E2 response (Abot et al, 2013 ; Adlanmerini et al, 2014).

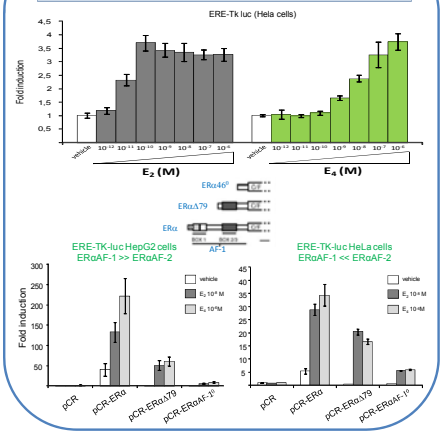
Comparison of the ERα LBD structure and of the coactivator interaction after E_2 and E_4 binding.



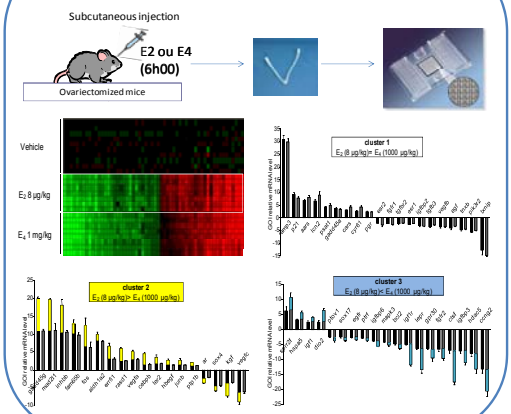
Ki = 0.2nM

Ki = 30.8 nM

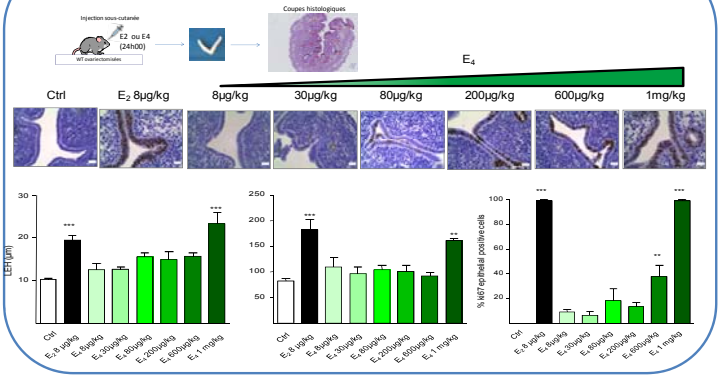
Respective roles of ERα AF-1 and AF-2 in the transcription activity of E_4



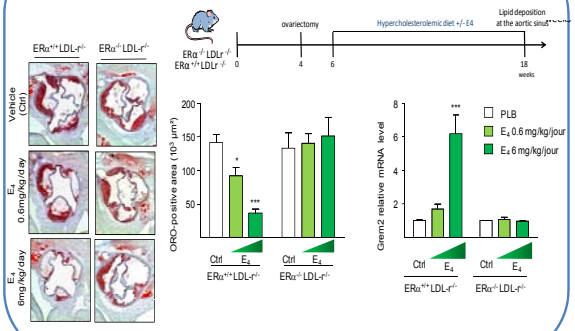
Impact of acute E_4 treatment on uterine gene expression



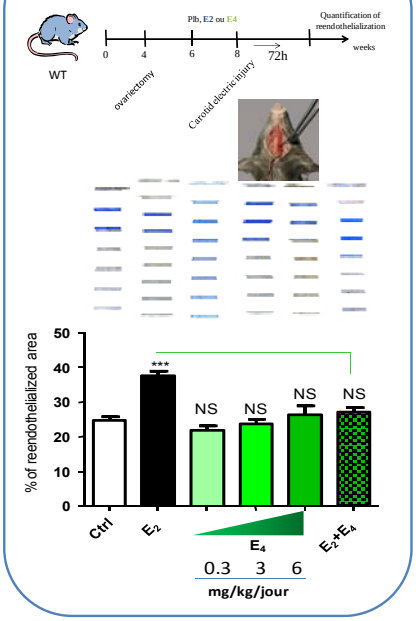
Impact of acute E_4 treatment on epithelial proliferation



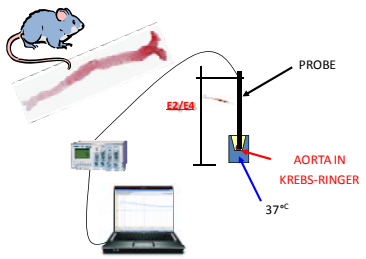
E_4 induces an atheroprotective effect in an ERα-dependent manner



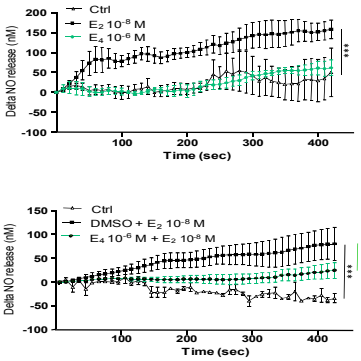
E_4 fails to accelerate endothelial healing.



E_4 fails to increase endothelial NO production



Recording of electric current genertated by oxidation of NO



Conclusion

✓ E4 is less potent than E2 to activate estrogen receptor alpha (ERα , but a high dose is able to modulate the transcriptional activity of ERα in the uterus, the proliferation of endometrial epithelium and to prevent atheroma. In contrast, E4 was not only devoid of effects on endothelial healing and NO production, but it antagonized these E2 effects that are purely membrane ERα-dependent.

✓ This original profile of ERα activation allows to characterize E4 as a selective ER modulator which could have medical applications that should now be considered further.