Effect of selective ablation of endothelin A receptor in the granulosa cells on the fertility
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Endothelin-2 (ET-2) is highly expressed in the granulosa cells (GCs) of periovulatory ovary. Previous experiments demonstrated that endothelin receptor antagonist inhibits ovulation in rodents. In the ovary ET-2 is expressed only in the GCs, while endothelin receptors are expressed in a variety of ovarian cell types. This study was designed to determine the significance of the expression of endothelin A receptor (ETA, a protein product of Ednra gene) in the granulosa cells. Two lines of GC-specific Ednra knockout mice were generated by crossing breeding Ednra-flx/flx mice with either Amhr2Cre or Cyp19Cre mice which express Cre recombinase under the anti-Mullerian hormone receptor promoter and the aromatase promoter, respectively. While both transgenic mice express Cre in the granulosa cells, the Cyp19Cre mouse were expected to have a higher rate of Ednra deletion than the Amhr2Cre mouse. Reproductive function of the resulting mice was measured by their fertility and litter size (number of pups per mouse and pups per litter) after breeding the GC-specific Ednra knockout female mice with proven males. Ednra deletion driven by either Amhr2Cre mice (GC-EdnraKO-Amhr2) or Cyp19Cre mice (GC-EdnraKO-Cyp19) reduced fertility (57.9\% in GC-EdnraKO-Amhr2 and 42.3\% in GC-EdnraKO-Cyp19 compared to 76.5\% in WT, p = 0.22 and p = 0.02, respectively). In addition, GC-EdnraKO-Amhr2 and GC-EdnraKO-Cyp19 mice had significantly smaller litter size as well (3.55 ± 0.45 and 4.56 ± 0.53 vs 7.08 ± 0.48 in WT, p < 0.01 for each). Taken together, this study demonstrates that ETA in granulosa cells plays an important role for female fertility in mice.


Negative allosteric modulation of endothelin ETA receptor function in resistance arteries
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Long-lasting arterial contractions induced by endothelin-1 (ET-1) are caused by tight binding of ET-1 to ETA receptors. Slow dissociation of ET-1 from ETA receptors suggests that i) an endogenous system effectively counteracts an otherwise continuously active contractile system and ii) inhibitors of contractile mechanisms or stimuli of relaxing mechanisms must induce long-lasting effects in order to effectively counteract the ET system. In isolated rat mesenteric resistance arteries, we studied these potentially effective pharmacological approaches. Calcitonin gene-related peptide (CGRP) only slightly reduced arterial sensitivity to ET-1 but terminated ET-1-induced vasospasms by promoting dissociation of ET-1/ETA receptor complexes. The CGRP effects were mediated by CGRP receptors and not mimicked by stimuli of mediators downstream of G\textalpha-proteins such as adenylate cyclase (AC), but rather were blocked by Q/γ- inhibition. Stimuli of AC such as forskolin and isoproterenol did produce a readily reversible relaxation of ET-1-induced vasospasms without affecting the initial contractions. The poorly reversible stimulator (Bay412272) and activator (Bay602770) of soluble guanylate cyclase produced long-lasting relaxations, also without inhibiting the initial ET-1-induced contractions. Rho-kinase inhibition (OH- fasudil) was without effect, TRPC3 or L-VOCC inhibition (Pyr3 and felodipine, respectively) only relaxed ET-1-induced vasospasms and PLC inhibition (U73122) strongly reduced contractile responses to ET-1 and relaxed the vasospasms. These results suggest that long-acting physiological and functional antagonists can effectively relax ET-1-induced vasospasms. Therefore, these systems can provide an alternative for current ETA antagonists which do not display this beneficial characteristic. This study was performed within the framework of TI Pharma projects T2-301 and T2-108.