Endothelin-2 induce ovulation by constricting ovarian follicle via EDNRA-mediated pathway

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Endothelin 2 (EDN2) induces follicular rupture for ovulation by constricting periovulatory follicles.We hypothesized that EDNRA expressed in the smooth would directly mediate the EDN2-induced contractile response.To test the hypothesis, we induced a selective null mutation of endothelin receptor type A (EDNRA) gene specifically in the smooth muscle cells of premature mice. Floxed EDNRA($EDNRA^{flox/flox}$) mice were cross bred with $SMA^{Cre}ER^{T2}$mice that express Cre recombinase specifically in the smooth muscle cells upon tamoxifen (TAM)injection in vivo. Through this breeding strategy, we produced two genotypes, $EDNRA^{flox/flox}SMA^{Cre}ER^{T2}$ and$EDNRA^{flox/flox}$. Upon TAM injection, null mutation was induced in the $EDNRA^{flox/flox}SMA^{Cre}ER^{T2}$ mice while EDNRA gene was intact in the $EDNRA^{flox/flox}$ mice. These mice were injected withTAM or vehicle (oil) at the age of 21 days after birth. TAM (0.5 mg/mouse/day) or oil was injected for 5 consecutive days. Then the animals were given 3-day long rest (no injection) before ovulation was induced by injecting themwith pregnant mare's serum gonadotropin (PMSG; 5 IU/mouse) and human chorionic gonadotropin (hCG; 5 IU/mouse).Eighteen hours after hCG injection, the mice were euthanized by CO_2 overdose followed byassessment of ovulatory capacity by counting the numbers ovulated oocytes and of contractile response to EDN2(50 mg/L) by measuring isometric tension.Number of ovulated oocytes were significantly lower $EDNRA^{flox/flox}SMA^{Cre}ER^{T2}$ mice that received TAM injection compared to oil treated ones (7.6± 3.2 oocytes/ovary in TAM group vs. 34.5±6.3 in oil group, $p<0.05$, n=8). In the $EDNRA^{flox/flox}$ mice, TAM did not reduce ovulatory capacity (15.4±8.4 oocytes/ovary in TAM group vs. 30.8±4.1; $p<0.05$, n=5). Contractile force was not increased after EDN2 treatment in the TAM-injected $EDNRA^{flox/flox}SMA^{Cre}ER^{T2}$ mouse ovary, not in the oil-injected mice.In conclusion, the results demonstrated that EDNRA mediates endothelin induced ovarian constriction.