Neurotensin Mediates Ovulation in Mouse Ovarian Follicles In Vitro

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Neurotensin (NTS) is a small peptide produced in the ovarian follicle. The ovulatory gonadotropin surge rapidly increases follicle expression of *NTS* mRNA in mice, rats, monkeys, and women. Inactivation of NTS within the follicle via immunoabsorption reduces ovulation in monkeys, implicating NTS as a paracrine mediator of ovulation. NTSR1, NTSR2 and SORT1 have been identified as functional NTS receptors. We hypothesize that NTS stimulates ovulation through one or more NTS receptor(s), and that blockade of NTS action at one or more NTS receptors will decrease ovulation. A model of in vitro mouse follicle culture was used in which ovaries were removed from female 32-39-day-old C57BL/6 mice. Secondary follicles were dissected from the ovary and cultured individually in follicle stimulating hormone-containing media. After four days, follicles that increased in diameter by at least 40% and remained circular and intact were treated with or without human chorionic gonadotropin (hCG) to stimulate ovulatory changes. Total RNA was collected from follicles at 0, 4, 8, and 12 hours treated with or without hCG, and mRNA for *NTS* and NTS receptors was quantified via qPCR (n=4 per treatment/time). hCG rapidly and transiently increased follicle expression of *NTS* mRNA, with levels peaking quickly at 4 hours and then decreasing at 8 and 12 hours ($p<0.05$). Follicular cells expressed mRNA for NTS receptors *NTSR1* and *SORT1* at all time points with and without hCG. *SORT1* mRNA was decreased at 4, 8, and 12 hours post-hCG ($p<0.05$) while *NTSR1* mRNA remained constant. *NTSR2* mRNA was not detected. In a separate experiment, follicles were treated with or without hCG for 18 hours to induce ovulation and then assessed via microscopy for follicle rupture, oocyte release, cumulus expansion, and luteinization. Media was assayed for progesterone and prostaglandin E2 accumulation by ELISA. Without hCG, ovulation was low (0-10% of follicles). hCG effectively stimulated ovulatory events after 18 hours (74-80% of follicles), including follicle rupture, release of a cumulus-oocyte-complex, luteinization of follicle cells, and increased media levels of progesterone ($p<0.05$ at 12, 18 hours; n=10) and prostaglandin E2 ($p<0.05$ at 18 hours; n=10). To determine if NTS receptor activation is important for ovulation, day 4 follicles were pre-treated for 1 hour before hCG administration with or without the general NTS receptor antagonist SR142948. In the presence of hCG, SR142948 resulted in a reduced rate of ovulation (45%, $p<0.05$; n=5), indicating that NTS action at one or more NTS receptors is important for ovulation. To determine which NTS receptors are involved in ovulation, follicles were pre-treated with either the NTSR1-selective antagonist SR48692 or the SORT1-selective antagonist Spadin for 1 hour before hCG administration. Blockade of either NTSR1 or SORT1 significantly reduced the rate of ovulation (SR48692 48%, n=3 and Spadin 58%, n=4; $p<0.05$) compared to hCG alone. Media progesterone and prostaglandin E2 in the presence of hCG was not affected by any of the antagonists. These data support the concept that locally-produced NTS is a paracrine factor which promotes ovulation in multiple mammalian species. The present findings are the first demonstration that NTSR1 and SORT1 are key receptors which mediate NTS-stimulated ovulatory events. Ultimately, NTS receptors may be useful therapeutic targets to promote ovulation in infertile women or reduce ovulation as a non-hormonal contraceptive. This work was supported by EVMS and NIH HD097675 to DMD and TEC.