A critical test of the hypothesis that ultra-short loop feedback via GnRH receptors coordinates pulsatile GnRH secretion. Charles M. Muller1, Kathryn Swanson1, Ulrich Boehm2, Teresa H. Horton1, and Jon E. Levine1. 1Department of Neurobiology and Physiology, Northwestern University, Evanston, IL, 2Center for Molecular Neurobiology, University of Hamburg, Hamburg, Germany

The pulsatile release of gonadotropin releasing hormone (GnRH) from neurons in the hypothalamus is essential for reproduction, however, the mechanism for this coordinated pulsatile release has not yet been identified. The diffuse arrangement of GnRH neurons stands in stark contrast to the coordinated release of the hormone. One proposed mechanism is an ultra-short feedback system within the hypothalamus that coordinates the pulses (1). Such a feedback system would involve the activation of GnRH receptors (GnRHR) in GnRH neurons themselves or in other cells that interact with GnRH neurons to coordinate the secretion of GnRH. We tested the hypothesis that cells in the hypothalamus containing GnRH receptors were part of an ultra-short loop feedback system.

To test this hypothesis, a novel mouse model was used in which cells expressing GnRHR were ablated using CRE-LOX technology. Mice in which a gene coding for diphtheria toxin (DTA) was flanked by LOX P sites (R-26-DTA mice) (2) were crossed with mice expressing CRE under control of the GnRHR promoter (GnRHR-internal ribosome entry site-Cre (GRIC mice)) (3). The presence of both CRE and LOX results in CRE activation allowing the cells to express DTA which kills the cells. This cross results in mice with selective ablation of cells expressing GnRH receptors (3). Groups of R26-DTA/GRIC (KO) mice were prepared for experiments to monitor GnRH release with GRIC or R-26-DTA mice serving as controls (WT). Adult females (>8 weeks) were fed a low phytoestrogen diet (Harlan-Teklad 2916) for two weeks prior to surgery and throughout the experiment. Six KO and 2 WT mice were implanted with a guide cannula directed toward the median eminence of the brain using stereotaxic coordinates (-1.94 AP, -0.25 LM, -4.9 DV relative to Bregma). After one week, mice were subjected to microdialysis to extract GnRH. Microdialysis was performed with artificial cerebrospinal fluid (aCSF) (flow rate 1.5 µl/min) with samples collected every 5 min for 3 hr. Mice were then dialyzed with KCl for 30 min, followed by aCSF for 30 min, again with samples collected every 5 min. GnRH content was measured by RIA.

The KO mice clearly exhibited pulsatile GnRH secretion. Although additional samples from WT animals of similar genetic background need to be collected, these data suggest that communication among cells via GnRH receptors is not required for the coordinated secretion of GnRH pulses.

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