Immortalized Ovine KNDy Neuronal Cell Lines Reveal Mechanisms Underlying Regulation of Reproduction by Sex Steroids and Seasonal Cues

Rebekka Toyoizumi[1], Christopher Y. Markgraf[1], Alia Starman[1], Blake L. Lee[1], Roxanne Bahn-Bales[1], Charles Estill, DVM[1], Charles E. Roselli, PhD[2], Patrick E. Chappell, PhD[1].

[1]Oregon State University, Corvallis, OR, USA, [2]OR Health Science University, Portland, OR, USA.

The multiphenotypic hypothalamic KNDy neurons play a critical role in the neuroendocrine regulation of reproduction via their stimulation of GnRH release, and are requisite for fertility and pubertal progression. *In vivo* evidence in mouse models has accumulated identifying the Arcuate population of Kisspeptin neurons in mediating predominantly negative feedback effects of gonadal steroids, and their ability to synthesize and secrete kisspeptin (KP), neurokinin B (NKB/Tac3), and dynorphin (Dyn) implicates them as crucial regulators of GnRH pulse generation.

We previously generated murine immortalized arcuate KNDy neuronal cell lines to explore molecular mechanisms underlying the steroid hormone regulation of these cells, and in the current study, we characterize novel immortalized ovine neuronal cell line model of KNDy (oKNDy) cells derived from fetal female sheep brain. Clonal lines were derived from isolated mediobasal hypothalamic explants using lentiviral infection of neurons with plasmids encoding SV40 large T-antigen. These neurons express ovine *kiss1*, *tac3*, and *pdyn*, as well as steroid receptors *esr1*, *esr2*, and *pgr*. Similar to KNDy neurons *in vivo*, receptors for Tac3 (*tacr3*) and Dyn (*oprk1*) were also found to be expressed in our oKNDy cells. Further, low physiological concentrations (5-50pM) of 17β-estradiol (E2) were found to significantly repress *kiss1* and *tac3* expression, while having no effect on *tacr3*. Additionally, concentrations of E2 and progesterone (P4) together approximating varying estrous cycle levels modulated *kiss1* and *tac3* expression. While cells exposed to higher E2 levels exhibited greater *kiss1/tac3* repression, P4 exposure alone revealed stimulation of expression with increasing P4.

Since these neurons were derived from the short-day seasonally breeding sheep, they can also be used to explore how circannual timing cues derived from the pituitary pars tuberalis may exert gating signals on the reproductive axis as previously modeled *in vivo*. Consistent with this, we have found high levels of thyroid hormone receptor α (*thra*) expression in our oKNDy neurons, and found *kiss1/tac3* expression inhibited by exogenous levothyroxine, suggesting they can respond to locally-synthesized T3. In conjunction with the above, we have also generated a *kiss1*- and *tac3*-expressing neuronal line from the ovine preoptic area (oPOA), which exhibit different morphology and E2-responsiveness from our oMBH-derived neurons. We are continuing to characterize these ovine neuronal cells in culture in order to provide insight into GnRH pulse generation and construct molecular models of neuronal responsiveness to steroids and other regulatory inputs.