## AuPS/ASB Meeting - Newcastle 2007

## Symposium: Endocrinology, reproduction and foetal development

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Chair: Chen Chen, Iain Robinson

## New kids on the block: RF-amides and neuroendocrine control of reproduction

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The reproductive process is driven by the secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus, which stimulates the synthesis and secretion of gonadotropins from the pituitary gland. The reproductive neuroendocrine system is, in turn, regulated by classical feedback exerted by gonadal steroids but GnRH cells do not possess the appropriate receptors. Accordingly, other neuronal systems in the brain, that do express receptors for reproductive steroids, relay information to GnRH cells. In recent years, it has emerged that cells in the hypothalamus that produce kisspeptin are provide a major input to the GnRH cells. Kisspeptin belongs to a family of peptides with a common C-terminal RF-amide motif. In the sheep brain, kisspeptin cells are found in the arcuate nucleus predominantly and are upregulated immediately prior to the preovulatory surge in GnRH (Estrada *et al.*, 2006). The kisspeptin cells express estrogen and progesterone receptors and respond appropriately to castration and sex-steroid treatment (Smith *et al.*, 2007; Smith & Clark, 2007). The kisspeptin cells also produce neurokinin B and dynorphin, two other regulators of GnRH cells (Goodman *et al.*, 2007). Administration (i.v.) of kisspeptin to seasonally anestrous ewes causes ovulation, further indicating that the peptide is a key regulator of reproduction (Caraty *et al.*, 2007). These data and information from other species indicate that kisspeptin cells provide a major postitive input to GnRH cells.

More recently, another RF-amide peptide has emerged as a major regulator of the reproductive neuroendocrine system. This is gonadotropin inhibitory hormone (GnIH), which is produced in the paraventricular nucleus and the dorsomedial nucleus of the hypothalamus. Whereas the function of this peptide has been expounded in birds, data has been lacking for a function in mammals. In the ovine brain, cells producing GnIH project to the secretory zone of the median eminence, suggesting secretion into the hypophysial portal system to act on the pituitary gland. GnIH potently inhibits GnRH-stimulated gonadotropin secretion from gonadotropes *in vitro* and *in vivo*. The peptide blocks GnRH-stimulated elevation in intracellular free calcium. Recent work on the function of these RF-amide peptides prompts a revision of the neuroendocrine control of reproduction.

Estrada KM, Clay CM, Pompolo S & Clarke IJ. (2006) *Journal of Neuroendocrinology*, **18:** 806-9.

Smith JT, Clay CM, Caraty A & Clarke IJ. (2007) *Endocrinology*, **148:**1150-7.

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Caraty A, Smith JT, Lomet D, Ben Said S, Morrissey A, Cognie J, Doughton B, Baril G, Briant C & Clarke IJ. (2007) *Endocrinology*, http://endo.endojournals.org/cgi/rapidpdf/en.2007-0554v1

## Involvement of TTX-resistant $Na^+$ currents and protein kinase C in the action of GHRH on primary cultured somatotropes from GH-GFP transgenic mice

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Growth hormone (GH) secretion is primarily mediated by two hypothalamic hormones, GH-releasing hormone (GHRH) and somatostatin. It is well-established that GHRH depolarizes the cell membrane of somatotropes and increases $Ca^{2+}$ influx through voltage-gated $Ca^{2+}$ channels, leading to an increase in intracellular free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) and GH secretion. Three major cation channels in somatotropes, $Ca^{2+}$, and $Na^+$, are involved in the regulation of cell excitability which, in turn, regulate GH secretion. It has been suggested that GHRH increases the membrane $Na^+$ permeability *via* $Na^+$ channels, which are not blocked by tetrodotoxin (TTX-resistant or TTX-R) but sensitive to cAMP levels, leading to a depolarization of the membrane and $Ca^{2+}$ influx (Kato & Sakuma, 1997). This TTX-R $Na^+$ channel has not been characterized in somatotropes to date. In this study, we demonstrate the presence of TTX-R $Na^+$ current and its modification by GHRH in Green Fluorescent Protein (GFP)-GH transgenic mice somatotropes, using the nystatin-perforated whole-cell patch-clamp recording configuration. The TTX-R $Na^+$ current was recorded from a holding potential of -70 mV in the presence of $Ca^{2+}$, $K^+$, and TTX-sensitive $Na^+$ channel blockers; tetraethylammonium (20 mM), $Co^{2+}$ (3 mM), and TTX (1 μM), respectively, in bath solution. GHRH (100 nM) was applied directly onto the cell and it caused a significant increase in the TTX-R $Na^+$ current, which was reversible with removal of GHRH. The GHRH-induced increase in TTX-R $Na^+$ current was, however, not affected by cAMP antagonist Rp-cAMP (100 μM), PKA inhibitor KT5720 (0.1 μM) or H89 (0.1 μM). In addition, the GHRH-induced increase in TTX-R $Na^+$ current was not affected by elevated cAMP levels; 8-bromo cAMP (0.1 mM), forskolin (1 μM, adenylyl-cyclase activator) and IBMX (0.5 mM, phosphodiesterase inhibitor), although these agents alone increased TTX-R $Na^+$ current, that is, in the absence of GHRH. U-73122 (5 μM, a PLC inhibitor) totally abolished the TTX-R $Na^+$ current response to GHRH. PKC inhibitors, Gö-6983 (1 μM) and chelerythrine (3 μM) also blocked the effect of GHRH. PDBu (phorbol dibutyrate, 0.5 μM, a PKC activator) increased TTX-R $Na^+$ current, but additional GHRH had no further effect on the current. These results suggest that the GHRH-induced increase in the TTX-R $Na^+$ current in mouse somatotropes is mediated by the PKC system. An increase in the TTX-R $Na^+$ current may depolarize the membrane, enhance $Ca^{2+}$ influx, and lead to GH secretion from somatotropes.

Kato M & Sakuma Y. (1997) *Endocrinology*, **138:** 5096-100.

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## Imaging and manipulating the growth hormone axis

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Pituitary growth hormone (GH) is released in a pulsatile fashion in response to stimuli from its hypothalamic regulators. For efficient generation of hormone pulses, both the hypothalamic mechanisms and the pituitary target cells need to be highly coordinated in their secretory activity. This is particularly important for the GH axis since the physiological responses in target tissues depend on the pattern of GH exposure, as well as on the amount of GH released. Recent strudies have shown that the pituitary GH cell populations are highly dynamic and show a remarkable degree of plasticity, with both cell number and hormone reserves varying in response to demands at different stages of life. In order to study this, we are exploiting a variety of genetic approaches to image, manipulate or ablate single or multiple populations of hypothalamic or pituitary cells, either during development or postnatally. Studies of mice with disruption of genes involved in pituitary development have shed light on mechanisms underlying similar problems in children with pituitary deficits and have led us towards the identification of a precursor cell population in the adult pituitary gland. We have also targeted fluorescent proteins to cytoplasmic or secretory granule compartments in the GH and prolactin (PRL) axes, allowing the application of a variety of fluorescence imaging techniques to study the fate of these hormones from the single vesicle level, to the entire pituitary population of cells, *in vitro*, *ex vivo* and even *in vivo*. By targeting transgene products that selectively alter specific cellular functions (*e.g.*, receptor signaling, ion-transport, RNA stability, vesicle packaging) we can manipulate selectively, populations of neuroendocrine and pituitary cells. These provide new models of somatotroph loss and compromised pituitary function, that extend our understanding of pituitary endocrine deficits, and enable us to test more specific novel therapeutic interventions.

# Growth hormone precursor cells: identification, enrichment, transplantation and differentiation

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Growth hormone(GH) secreted by the somatotrope cells of the pituitary gland is critically essential throughout life, regulating growth, bone density, the cardiovascular system and the metabolism of proteins, carbohydrates and lipids. Deficiency in GH is a significant clinical problem that affects both adults and children. The current therapy of GH replacement by injection does not simulate the natural physiologically controlled release from somatotrope cells. Little is known on the mechanism and maintenance of correct somatotrope numbers in a normal pituitary gland. Interestingly the pituitary has an enormous capacity for the expansion and complete restoration of the GH secreting population. Past studies have implicated a resident stem/precursor-like cell, however, this cell type was never identified.

Recently our laboratory was the first to report the identification and characterisation of a resident pituitary precursor cell that shares characteristics with stem cells. This cell type is rare, has clonogenic properties forming a heterogeneous colony from a single cell, has high proliferative potential, expresses cell surface stem cell-related antigens and shows the capacity to differentiate into GH cells. We termed this cell type **P**ituitary **C**olony **F**orming **C**ell or PCFC. The expansion and differentiation characteristics of PCFCs make them ideal for potential use in a cell based therapy for GH deficiency.

We have developed a protocol to obtain highly enriched populations of PCFCs using a combination of cell surface antigen markers and the ability of PCFCs to import the fluorescent di-peptide β-Ala-Lys-Nε-7-amino-4-methylcoumarin-3-acetic acid (AMCA). To test PCFC potential *in vivo* we used a tissue engineering model specifically developed by our laboratory to grow transplanted tissue/cells for repair. This model involves a vascularized microchamber system in the mouse offering the advantage of circulating blood to supply the cells with nutrients to maintain survival. PCFCs implanted into the microchamber both survived and differentiated into GH cells *in vivo*. Our studies show that the PCFC microchamber system has the potential to be developed into a GH secreting organoid.