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**EFFECT OF CALCITONIN GENE-RELATED PEPTIDE ON GnRH mRNA EXPRESSION IN THE GT1-7 CELL**J. Kinsey-Jones<sup>1</sup>, X. Li<sup>1</sup>, J. Bowe<sup>1</sup>, S. Brain<sup>2</sup> and K. O'Byrne<sup>1</sup><sup>1</sup>Division of Reproductive Health, Endocrinology and Development, Kings College London, London, UK and <sup>2</sup>Centre for Cardiovascular Biology, Kings College London, London, UK

Calcitonin gene-related peptide (CGRP) has recently been shown to induce a profound suppression of the hypothalamic gonadotrophin-releasing hormone (GnRH) pulse generator, resulting in an inhibition of pulsatile luteinising hormone (LH) secretion in the rat (Li *et al.*, 2004). The aims of the present study were, (i) to determine the presence of the CGRP receptor subunits, receptor activity modifying protein-1 (RAMP-1) and calcitonin receptor like receptor (CL), both of which are required for functional activity, in the GT1-7 cell line, an established *in vitro* model for hypothalamic GnRH neurones (Mellon *et al.* 1990), and (ii) to test the hypothesis that CGRP acts directly on the GnRH neurone to suppress GnRH mRNA expression. GT1-7 cells were grown in Dulbecco's Modified Eagle Medium supplemented with 10% fetal calf serum, 4.5 mg/ml glucose and antibiotics and maintained at 37°C in an atmosphere of 5% CO<sub>2</sub>. Cells were harvested on ice and total RNA was extracted. The mRNAs for both CL and RAMP-1 receptor subunits were detected by reverse transcription PCR (RT-PCR) in the GT1-7 cell. GT1-7 cells were cultured with different concentrations of CGRP ranging from 10<sup>-6</sup> to 10<sup>-12</sup> M, and for a series of time points at 6, 12, 24 and 36hrs. In addition, CGRP (10<sup>-8</sup> M) was also co-administered with a CGRP receptor specific antagonist CGRP<sub>8-37</sub> (10<sup>-6</sup> M). GnRH mRNA levels were investigated by quantitative real time RT-PCR. All treatments were run in triplicate and experiment were repeated three times. The levels of GnRH mRNA expression were significantly reduced after treatment with CGRP at 6, 12 and 24 hrs, with maximum inhibition at 24 hrs. CGRP resulted in a dose dependent suppression of GnRH mRNA expression, with the most effective dose at 10<sup>-8</sup>M CGRP, resulting in 70.5±6.8% (mean±SEM, P<0.05 tested with one way ANOVA) suppression. Co-culture with CGRP and the selective receptor antagonist CGRP<sub>8-37</sub> was able to completely reverse the inhibitory effect of CGRP on GnRH mRNA expression. These results demonstrate for the first time the presence of the CGRP receptor in GT1-7 cells, and suggest that CGRP directly regulates GnRH gene expression via CGRP receptors, which might play a physiological role in the regulation of the GnRH pulse generator.

Li, X. F., Bowe, J. E., Mitchell, J. C., Brain, S. D., Lightman, S. L., & O'Byrne, K. T. (2004) Stress-induced suppression of the gonadotropin-releasing hormone pulse generator in the female rat: a novel neural action for calcitonin gene-related peptide. *Endocrinology* 145, 1556-1563.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

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**Effects of the potent hop-derived phytoestrogen, 8-prenylnaringenin, on the reproductive neuroendocrine axis**J. Bowe<sup>1</sup>, J. Kinsey-Jones<sup>1</sup>, X. Li<sup>1</sup>, S. Brain<sup>2</sup> and K. O'Byrne<sup>1</sup><sup>1</sup>Division of Reproductive Health, Endocrinology and Development, King's College London, London, UK and <sup>2</sup>Centre for Cardiovascular Biology, King's College London, London, UK

The phytoestrogen, 8-prenylnaringenin (8-PN), is the most potent phytoestrogen discovered to date. Derived from hops, it is present in dietary supplements currently marketed for natural breast enhancement, though little is known about efficacy rates or other effects (Coldham & Sauer, 2001). 8-PN is also of potential interest in the treatment of menopausal symptoms and diseases involving angiogenesis. It is known that various phytoestrogens produce inhibitory effects on gonadotrophin secretion in both humans and animals (McGarvey *et al.* 2001). The aims of the present study were to investigate effects of 8-PN on the reproductive axis, both *in vivo*, by examining effects of 8-PN on LH pulses, and *in vitro*, through studying the effects of 8-PN on GnRH mRNA expression in the GnRH cell line, the GT1-7 cells.

Ovariectomized (ketamine, 100 mg kg<sup>-1</sup>, ip) Wistar rats were chronically implanted with intravenous (iv) cannulae (ketamine, 100 mg kg<sup>-1</sup>, ip). Blood samples (25µl) were collected at 5 min intervals for 8hrs for the detection of LH. After 2hr sampling either 17β-oestradiol (E2, 0.2µg bolus followed by 0.2µg/h for 6h) or 8-PN (20µg bolus followed by 20µg/h for 6h) were infused intravenously (n=7). Thirty minutes prior to the end of sampling GnRH was administered (500ng/kg, iv bolus injection) to test pituitary responsiveness. GT1-7 cells were grown in Dulbecco's modified eagle medium supplemented with 10% fetal calf serum, 4.5 mg/ml glucose and antibiotics and maintained at 37°C. GT1-7 cells were cultured with 8-PN concentrations ranging from 10<sup>-5</sup>M to 10<sup>-8</sup>M, then harvested on ice and total RNA was extracted. GnRH mRNA levels were investigated by quantitative real time RT-PCR.

Both E2 and 8-PN, caused a profound reduction in LH pulse frequency (68.0±7.3% and 148.7±18.1% increases in LH inter-pulse interval, respectively, mean±sem) and amplitude (33.6±4.3% and 47.4±6.7% decrease in LH pulse amplitude respectively) compared with controls (p<0.05). Furthermore 8-PN blocked pituitary response to GnRH. In the GT1-7 cells, 8-PN treatments induced a dose dependent suppression in GnRH mRNA expression (63.9±7.2% at 10<sup>-5</sup>M 8-PN).

These results suggest that suppression of pulsatile LH secretion by 8-PN may involve a hypothalamic and pituitary site of action. Further a direct inhibitory action at the level of the GnRH neurone is also implicated.

Coldham, N.G., Sauer, M.J. (2001) *Food Chem Toxicol.* 39(12):1211-24

McGarvey, C., Cates, P.S., Brooks, A.N., Swanson, I.A., Milligan, S.R., Coen, C.W., O'Byrne, K.T. (2001) *Endocrinology.* 142(3): 1202-08

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