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Effect of age & pregnancy on the expression of melanocortin receptors 4 & 5 in the mouse female reproductive axis

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Introduction: There are five melanocortin receptors (MC_{1-5}): these are G protein-coupled receptors expressed in the central nervous system and in peripheral tissues. MC_4 and MC_5 have roles in controlling appetite, immuno-modulation, exocrine function, erectile dysfunction and grooming behaviour (1). The melanocortin receptor accessory proteins (MRAP1 and 2) influence MC receptor transport. Proopiomelanocortin (POMC) is the precursor for the alpha-melanocyte stimulating hormones and adrenocorticotrophic hormone, which bind to MC_4 and MC_5 receptors (1).

Aims: 1.To characterise MC_4 and MC_5 and MRAP1 distribution in the reproductive tissues of female mice (hypothalamus, pituitary gland, uterus and ovary); 2. To investigate if MC_4, MC_5 and MRAP1 expression changes with age or pregnancy.

Method: Virgin C57BL/6 female mice aged 2, 6, 9, 10 and 14 weeks and pregnant (aged 9 weeks plus 13 days *post coitus*) were sacrificed by schedule 1. Tissue RNA was extracted by TRIzol/RNA cleanup (Qiagen) and cDNA synthesised using SuperScript II reverse transcriptase (Thermoscientific). Appropriate reference genes were determined using GeNorm selection kit and software (PrimerDesign; Biogazelle respectively). The following genes were the most stable and used in SYBRgreen RTqPCR according to precisionPLUS master mix protocol (PrimerDesign for normalisation (2) of MC_4, MC_5 and MRAP1 expression: Qiagen primers).

Tissue	Reference gene 1	Reference gene 2
Hypothalamus	YWHAZ	CANX
Pituitary gland	EIF4A2	CANX
Ovary	GAPDH	ATP5B
Uterus	CYC1	RPL13A

Results: MC_4 and MC_5 were expressed in the hypothalamus, pituitary, ovary and uterus. MC_4 and MC_5 expression was higher in 2 weeks old mice in the hypothalamus (n=3, p=0.0056 and <0.0001) and uterus (n=3, p=0.0119 and <0.0001 respectively) compared to all other age groups using one-way ANOVA and Tukey's *post-hoc* test. Pregnancy did not affect either MC_4 or MC_5expression. MRAP1 was expressed in the hypothalamus, ovary and pituitary and was unaffected by age. MRAP1 expression was greater in the ovary and decreased in the hypothalamus of pregnant compared to non-pregnant mice (n=3, p<0.0001).

Conclusion: The role of the two MCs in the development of the hypothalamus and uterus in young

mice requires further investigation. The changes in MRAP1 expression with pregnancy could result in changes in the signalling ability of both MC_4 and MC_5since MRAP1 has been shown to alter cAMP production resulting from activation of these two receptors *in vitro* (3).

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