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Melanocortin 3 receptor in the testes of mice

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Melanocortin 3 receptors (MC3-R) are expressed in the brain, spinal cord and the periphery. The physiological roles of the peripheral receptors are unclear. Although it has been reported that MC3R is not expressed in the testes of adult mice (O'Shaughnessy *et al* 2003), "in house" difficulties using the male MC3-R null mice for breeding stimulated our interest in examining these animals more closely. The aims of this preliminary study were to determine if MC3-R is expressed in testes and to examine the histology of MC3-R null testes.

Testes from 4 wild type (C57 B1.6) and 4 MC3-R null (on a homogenous C57B16 background) mice (Getting 2006) were embedded in paraffin and serially sectioned (5 μm thick). The animals were not aged matched and varied from 8 to 16 weeks of age in this preliminary study. Immunohistochemistry was done using a previously characterized rabbit anti-MC3-R (Leoni *et al* 2008). Histological assessment was done on every 10th slide stained with haematoxylin and eosin. Three frames in one section on each stained slide were captured for image analyses. The diameter and the thickness of the cellular layer lining each seminiferous tubule in each frame were measured.

Immunopositive staining for MC3-R, consistent with it being associated with the cell membrane, was observed in cells of the interstitial tissue and in some cells within the seminiferous tubules. Within the seminiferous tubules occasional clusters of cells with very intense staining both within the cytoplasm and the nucleus were observed. No staining was observed in the MC3-R null tissue or in the absence of primary antibody. There was no difference in either the diameter or the thickness of the cellular layer within the seminiferous tubules between wild type and MC3-R null mice (diameter: wild type (n=4) = 97.2 $\pm$ 6.6 μm versus MC3R null (n=4) = 89.5 $\pm$ 3.0 μm, not significant $P > 0.05$; thickness: wild type (n=4) = 25.1 $\pm$ 1.0 μm versus MC3R null (n=4) = 23.8 $\pm$ 0.6 μm, not significant $P > 0.05$). There appeared however to be less cells lining the seminiferous tubules of the MC3-R null mice: in many tubules the usual arrangement of the Sertoli cells and developing germ cells was disrupted.

Testicular function in MC3-R null mice is disrupted and this appears to be due to a direct effect on the testes since MC3-R are expressed in the testes. Previous published work suggests a role for melanocortins in fetal testes but not in adult testes. The results of this preliminary study suggest that MC3R and its ligands may have a role in normal adult testicular function which remains to be elucidated.

Getting, S.J. *et al*. (2006). *FASEB J*; 20: 2234-2241

Leoni, G. *et al*. (2008). *FASEB J*; 22: 4228-4238

O'Shaughnessy, P.J. *et al*. (2003). *Endo*; 144: 3279-3284