

## Poster Abstract

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### **Role of N-linked glycosylation in setting the Ca<sup>2+</sup><sub>o</sub> sensitivity of the extracellular calcium-sensing receptor**

Brennan SC and Conigrave AD

*School of Molecular and Microbial Biosciences, University of Sydney, NSW, 2006, Australia*

The calcium-sensing receptor (CaR) is a class 3 GPCR that plays an important role in calcium metabolism and bone homeostasis. The receptor is activated not only by extracellular calcium (Ca<sup>2+</sup><sub>o</sub>), but also type-II calcimimetics and L-amino acids. The CaR has 11 putative N-linked glycosylation sites in the extracellular domain and at least 3 of these sites are required for cell surface expression [1]. In addition, the lectin Concanavalin-A promotes Parathyroid Hormone secretion [2], consistent with an inhibitory effect on the CaR. In the current study we used the enzyme Peptide N-Glycosidase F (PNGase F) to cleave N-linked sugars in CaR-expressing HEK293 cells (HEK-CaR) prior to loading with Fura-2 AM for analysis of Ca<sup>2+</sup><sub>o</sub>-dependent receptor activation. Pre-treatment of HEK-CaR cells with PNGase F strikingly right-shifted the concentration response curve for receptor activation. For control HEK-CaR cells the EC<sub>50</sub> value for Ca<sup>2+</sup><sub>o</sub> was 4.2 ± 0.2 mM (n = 3). However in HEK-CaR cells, pre-treated with PNGase F for 16 hours at 37°C the EC<sub>50</sub> for Ca<sup>2+</sup><sub>o</sub> was 7.3 ± 0.5 mM (n = 4). Remarkably, both the amino acid L-Phe and the calcimimetic R467 restored Ca<sup>2+</sup><sub>o</sub> sensitivity. The results indicate that N-glycosylation of the CaR modulates extracellular Ca<sup>2+</sup><sub>o</sub>-sensitivity in CaR-expressing cells.

1. Ray, K., et al., J Biol Chem, 1998. **273**(51): p. 34558-67.
2. el-Hajj Fuleihan, G., et al., Endocrinology, 1991. **128**(6): p. 2931-6.