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Running Head: Calcium signalling by Indo-1 flow cytometry assay

i. **Monitoring calcium-sensing receptor (CaSR) induced intracellular calcium flux using an Indo-1 flow cytometry assay**

ii. **Abstract** (1-2 paragraphs)

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This method describes the loading of cells with Indo-1AM to assess intracellular calcium flux by flow cytometry. Indo-1 is...

iii. **Key words** (5-10 words for referencing): Adaptor protein-2 sigma subunit, calcium homeostasis, calcium flux, G-alpha protein-11, hyper/ hypocalcaemia, parathyroid hormone.

1. Introduction

Calcium is the most abundant mineral in the human body and tight control of intra- and extracellular calcium concentrations is crucial for normal physiology. Extracellular free calcium concentrations are typically 1.1-1.3mM, and intracellular concentrations are ~100nM. The calcium-sensing receptor (CaSR) is the master-regulator of calcium homeostasis, detecting small changes in extracellular calcium concentrations and initiating intracellular signaling pathways and hormone release to alter calcium uptake, secretion or excretion and maintain calcium concentrations within the normal physiological range.

The CaSR is a G-protein coupled receptor (GPCR) that is primarily expressed on the cell surface of the chief cells of the parathyroid gland, apical and basolateral surfaces of renal tubular segments, and bone marrow stem cells, chondrocytes, osteoblasts, and osteoclasts,. Mutations of the CaSR result in disorders of extracellular calcium homeostasis. Thus, heterozygous CaSR loss-of-function mutations cause familial hypocalciuric hypercalcaemia type 1 (FHH1) and biallelic loss-of-function mutations cause neonatal severe hyperparathyroidism (NSHPT); gain-of-function mutations cause autosomal dominant hypocalcaemia type (ADH1). Furthermore, FHH and ADH type 2 are caused by loss- and gain-of-function mutations, respectively, in G-protein alpha 11 (Gα11), and FHH type 3 is due to loss-of-function mutations of the adaptor protein 2 sigma subunit 1 (AP2σ); Gα11 and AP2σ are CaSR-signalling partners. FHH is characterised by a lifelong elevation in serum calcium concentration, reduced urinary calcium excretion, and inappropriately normal circulating PTH concentrations. NSHPT commonly presenting in the neonatal period with extreme hypercalcaemia, failure to thrive, hypotonia, anorexia, constipation and, in some cases, respiratory

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