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**INTRACELLULAR TRAFFICKING OF CANNABINOID
RECEPTOR 1**

A THOROUGH CHARACTERISATION AND INVESTIGATION
INTO THE ROLE OF THE INTRACELLULAR POOL

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ABSTRACT

Cannabinoid Receptor 1 (CB₁), an abundant G-protein coupled receptor (GPCR) in the central nervous system, is currently of significant interest as a therapeutic target. The cellular control of receptor trafficking is intimately linked with drug effects, however in comparison with other GPCRs, the study of CB₁ trafficking is in its infancy. Although the existing literature suggests CB₁ should be classified as a “dual-fate” receptor, some conflicting evidence exists as to the conditions under which CB₁ recycles or degrades. Of particular interest is the widely noted intracellular pool which has been speculated to form part of a constitutive internalisation and recycling pathway.

This study performs a detailed quantification of CB₁ trafficking in four cell lines, one of which expresses CB₁ endogenously. A novel high-throughput immunocytochemistry-based approach is applied to quantitatively measure receptor trafficking. An important advance on previous studies is the use of a proteolytic method to directly quantitate intracellular receptors. Contrary to previous reports, the data suggests that CB₁ does not recycle following constitutive or agonist-induced internalisation but instead exhibits a primarily degradative phenotype. Evidence is obtained through antibody “live-feeding” protocols and the effects of protein synthesis inhibitors, among other approaches. In addition, the data suggests that the intracellular pool does not traffic to the cell surface and therefore does not contribute to CB₁ signalling via classical paradigms. The effects of Rab GTPase dominantly-acting positive and negative mutants on basal CB₁ localisation corroborate these results.

The findings of this thesis have significant implications for the interpretation of CB₁ biochemical studies and call for a revision of the currently held theories of CB₁ intracellular trafficking. The study provides a foundation for further mechanistic studies and may impact the design and application of cannabinoid therapeutics.

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ABBREVIATIONS

| | |
|---|--|
| ANOVA, analysis of variance | min, minutes |
| ATCC, American Type Culture Collection | M, mol/L (molar) |
| β_2 AR, β_2 -adrenergic receptor | MFR, mean fluorescence ratio |
| BSA, bovine serum albumin | NA, not applicable |
| cAMP, cyclic adenosine monophosphate | NFM, non-fat milk |
| CB ₁ , Cannabinoid receptor 1 | PCR, polymerase chain reaction |
| CB ₂ , Cannabinoid receptor 2 | PBS, phosphate-buffered saline |
| cDNA, complementary DNA | PBS-T, PBS with 0.2% Triton X-100 |
| CHX, cycloheximide | PFA, paraformaldehyde |
| ConA, Concanavalin A | <i>p</i> , <i>p</i> -value |
| D ₁ , Dopamine receptor 1 | pg., page |
| DMEM, Dulbecco's modified eagle's medium | PNGase F, peptide-N-glycosidase F |
| EC ₅₀ , half maximal effective concentration | Rab, Ras-like from brain |
| EGFP, enhanced green fluorescent protein | RT, room temperature |
| ER, endoplasmic reticulum | RM, repeated measures |
| FBS, fetal bovine serum | sec, seconds |
| FSM, full-serum media | SFM, serum-free media |
| g, grams | SR, SR 141716A |
| G-protein, GTP binding protein | $t_{1/2}$, half-life |
| GASP, GPCR-associated sorting protein | TBS-T, Tris Buffered Saline with 0.05% |
| GPCR, G-protein coupled receptor | Tween |
| h, hour | TIS, trypsin inhibitor from soybean |
| HA, haemagglutinin | TGVC, total grey value per cell |
| HEK, human embryonic kidney-293 | WIN, WIN 55212-2 |
| HU, HU 210 | wt, wild-type |
| kDa, kilodaltons | xg, times gravity |