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 (CB_1), 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 CB_1 trafficking is in its infancy. Although the existing literature suggests CB_1 should be classified as a "dual-fate" receptor, some conflicting evidence exists as to the conditions under which CB_1 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 CB_1 trafficking in four cell lines, one of which expresses CB_1 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 CB_1 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 CB_1 signalling via classical paradigms. The effects of Rab GTPase dominantly-acting positive and negative mutants on basal CB_1 localisation corroborate these results.

The findings of this thesis have significant implications for the interpretation of CB_1 biochemical studies and call for a revision of the currently held theories of CB_1 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

ATCC, American Type Culture Collection

β_2AR, β_2-adrenergic receptor

BSA, bovine serum albumin

cAMP, cyclic adenosine monophosphate

CB_1, Cannabinoid receptor 1

CB_2, Cannabinoid receptor 2

cDNA, complementary DNA

CHX, cycloheximide

ConA, Concanavalin A

D_1, Dopamine receptor 1

DMEM, Dulbecco's modified eagle's medium

EC_{50}, half maximal effective concentration

EGFP, enhanced green fluorescent protein

ER, endoplasmic reticulum

FBS, fetal bovine serum

FSM, full-serum media

g, grams

G-protein, GTP binding protein

GASP, GPCR-associated sorting protein

GPCR, G-protein coupled receptor

h, hour

HA, haemagglutinin

HEK, human embryonic kidney-293

HU, HU 210

kDa, kilodaltons

min, minutes

M, mol/L (molar)

MFR, mean fluorescence ratio

NA, not applicable

NFM, non-fat milk

PCR, polymerase chain reaction

PBS, phosphate-buffered saline

PBS-T, PBS with 0.2% Triton X-100

PFA, paraformaldehyde

p, *p*-value

pg., page

PNGase F, peptide-N-glycosidase F

Rab, Ras-like from brain

RT, room temperature

RM, repeated measures

sec, seconds

SFM, serum-free media

SR, SR 141716A

t½, half-life

TBS-T, Tris Buffered Saline with 0.05% Tween

TIS, trypsin inhibitor from soybean

TGVC, total grey value per cell

WIN, WIN 55212-2

wt, wild-type

x*g*, times gravity