INVESTIGATING THE THERAPEUTIC POTENTIAL OF THE CB1 CANNABINOID RECEPTOR IN HUNTINGTON'S DISEASE

Emma L. Daniel

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ABSTRACT

The degeneration of neurons in the striatum, cortex and other diffuse brain regions in Huntington's disease (HD) is a progressive process, leading to debilitating symptoms and ultimately death. Despite the identification of the *huntingtin* gene, whose genetic mutation underlies the disease, the pathophysiology of HD remains ill-defined and there are currently no drugs in clinical use which effectively delay the progression of the disease. Recently, the CB1 cannabinoid receptor (CB1R) has gained attention as a therapeutic target in neurodegenerative diseases such as Parkinson's, and investigations have also begun in animal models of HD. However, these studies have yielded mixed reports of the efficacy of cannabinoids in this context. This may relate to the finding that CB1R binding is dramatically reduced early in the human disease. This thesis undertook the investigation of the therapeutic potential of the CB1R through the development of a PC12 cell model of HD. This model was found to recapitulate expression and aggregation of mutant huntingtin, loss of CB1Rs early in the pathological process, and cell death.

Using the Alamar Blue assay, it was determined that the CB1R agonists HU210 and WIN55,212-2, produced a small but significant reduction in huntingtin-associated cell death (6-8%). This rescue was dependent upon the activation of the Gαi G-protein subtype and ERK 1/2, which was necessary but not sufficient to modulate cell survival. Conversely, compounds which increased cAMP formation exacerbated cell death and this correlated with enhanced formation of mutant huntingtin aggregates. Interestingly, the protective CB1R agonist HU210 also increased cellular aggregate load (6%). While the reduction of cell death by HU210 had been mediated by Gαi-coupled CB1Rs, the enhancement of aggregate formation was likely to have occurred through CB1Rs coupling to Gαs. The enhancement of huntingtin aggregation was generally associated with toxicity. Therefore the ability of the CB1R to promiscuously couple to Gαs, and this detrimental aggregation pathway, may limit its therapeutic usefulness and account for the modest survival effects described here. However, compounds which are selective for Gαi activation by the CB1R, or compounds which target protective downstream effectors, could represent novel therapeutic strategies to alleviate neurodegeneration in HD.

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ABBREVIATIONS

2-AG	2-arachidonyl glycerol
3NP	3-nitropropionic acid
6E	Ecdysone receptor-responsive promoter
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
BAY59-3074	3-[2-Cyano-3-(trifluoromethyl)phenoxy] phenyl 4,4,4-trifluoro-1-butanesulfonic acid ester
BDNF	Brain derived neurotrophic factor
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
cDNA	Complimentary DNA
CMV	Cytomegalovirus
CRE	cAMP response element
DEPC	Diethylpyrocarbonate
dNTP	Dinucleotide triphosphates
DSE	Depolarisation-induced suppression of excitation
DSI	Depolarisation-induced suppression of inhibition
DTT	Dithiothreitol
EcR	Ecdysone receptor
EGFP	Enhanced green fluorescent protein
Enk	Enkephalin
EPSP	Excitatory postsynaptic potential
ER	Endoplasmic reticulum
ERK	Extracellular signal regulated kinase
FAN	Factor associated with neutral sphingomyelinase activation
GABA	γ-aminobutyric acid
GIRK	G-protein coupled inwardly rectifying potassium channel
GPCR	G-protein coupled receptor
GPe	Globus pallidus externus
GPi	Globus pallidus internus

G-protein	GTP-binding protein
HA	Haemagglutinin
HAP1	Huntingtin associated protein 1
HAT	Histone acetyl transferase
HD	Huntington's disease
HDAC	Histone deacetylase
HEK	Human embryonic kidney
HIP1	Huntingtin interacting protein 1
HU210	(6aR,10aR)-9-(Hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydrobenzo[c]chromen-1-ol
IBMX	Isobutylmethylxanthine
IEG	Immediate early gene
IP3	Inositol triphosphate
IT15	Interesting Transcript 15
JNK	c-jun N-terminal kinase
KA	Kainic acid
LB	Luria-Bertani
MAPK	Mitogen-activated protein kinase
MEK	Mitogen-activated protein kinase kinase
mRNA	Messenger RNA
MSN	Medium spiny neuron
NADA	N-arachidonyl-dopamine
NADP	Nicotinamide adenine dinucleotide phosphate
NMDA	N-methyl-D-aspartic acid
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PGK	Phosphoglycerate kinase
PI3K γ	Phosphatidyl-inositol-3-kinase γ
PKA	cAMP-dependent protein kinase A
PKB	Protein kinase B
PLCβ	Phospholipase C β
PRD	Proline rich domain

polyQ	Polyglutamine
PPN	Pedunculopontine nucleus
PTX	Pertussis toxin
QA	Quinolinic acid
Rp-cAMPS	Rp-Adenosine-3',5'-cyclic monophosphorothioate
RT-PCR	Reverse transcriptase PCR
RXR	Retinoid X receptor
RyR	Ryanodine receptor
SCH23390	7-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-lH-3-benzazepine-7-ol maleate
SFM	Serum-free media
SKF38393	1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
Sp1	Stimulating protein 1
SR141716A	N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H- pyrazole-3-carboxamide
SRE	Serum response element
STN	Subthalamic nucleus
TFZ	Tebufenozide, N -tert-butyl-N'-(4-ethylbenzoyl)-3,5-dimethylbenzo-hydrazide
TNFR	Tumour necrosis factor receptor
WIN55,212-2	(R)-(+)-[2,3-dihydro-5-methyl-3[(4-morpholinyl)methyl] pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate salt]
YAC	Yeast artificial chromosome
Δ^9-THC	Δ^9-tetrahydrocannabinol