Investigation of the role of peroxisome proliferator-activated receptors in the endocannabinoid system of *Tetrahymena pyriformis*

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The human endocannabinoid system (ECS) modulates many vital physiological functions, bringing balance to everything from sleep, appetite, pain, inflammation, memory and mood. It comprises lipid endocannabinoids, e.g. Anandamide (AEA), which are synthesized on demand and bind to receptors (CB1, CB2, GPR55, TRPV1). Cannabis induces similar effects due to its phytocannabinoids, Δ9-tetrahydrocannabidiol (THC) and cannabidiol (CBD), binding to these receptors. Single-celled protists can respond to AEA, THC and CBD, often showing reduced motility and cell division yet they do not possess these cannabinoid receptors. This suggests they possess a rudimentary ECS but to date its complete nature and function are unknown.

The ciliate *Tetrahymena* possesses a suite of endocannabinoids (including AEA), with N-palmitoylethanolamide (PEA) and N-oleoylethanolamide (OEA) being two of the dominant compounds. In humans OEA modulates body weight and feeding while PEA modulates pain. Their natural ligand is the peroxisome proliferator-activated receptor alpha (PPARα) but this receptor has not been identified in the *Tetrahymena thermophila* genome. It may be that *Tetrahymena* possesses a 'PPAR-like' receptor or it might not possess one at all, suggesting that cannabinoids evolved long before their currently-recognized receptors and originally served other roles.

This study examines the potential role of 'PPARs' in the ECS of *Tetrahymena pyriformis* by examining cell death in the presence and absence of PPAR blockers. Cells were first incubated with agonists to the three PPAR types, i.e. OEA/PEA (PPARα), GW0742 (PPARβ), Rosiglitazone (PPARγ). Only GW0742 (IC50 12μM) and OEA (IC50 45μM) caused cell death at concentrations similar to those recorded for human cells. PEA required higher concentrations (IC50 180μM) suggesting that if this does bind to PPARα (like OEA) it affects something other than cell division; this could also apply to Rosiglitazone binding to PPARγ (IC50 180μM). Experiments are now underway examining cell death (at IC50) in the presence of PPAR blockers.