Neuroprotective Properties of the Coffee Component Eicosanoyl-5-hydroxytryptamide Assessed *in vitro*

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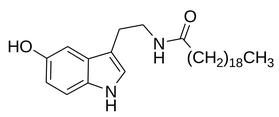
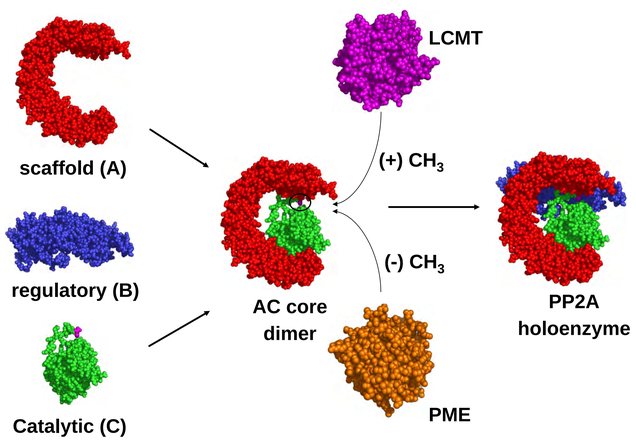
Abstract

Coffee is a complex mixture of more than eight hundred compounds with a variety of health benefits including reducing the risk of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. A coffee compound, unrelated to caffeine, called eicosanoyl-5-hydroxytryptamide has been identified as a regulator of the major serine/threonine phosphatase in the brain, protein phosphatase 2A (PP2A)[1]. In diseased tissue, PP2A exhibits reduced activity[2-3] and methylation of its carboxy-terminal tail[4]. Previous reports indicate eicosanoyl-5-hydroxytryptamide (EHT) is able to protect PP2A's methylation state and activity in vitro in addition to improving cognitive and motor function in animal models[1,5-7]. In order to further understand the role of EHT in neuroprotection we sought to evaluate its ability to combat oxidative injury, structural damage and chemical toxicity in neuronal cell cultures. Utilizing lipid peroxidation as a marker for oxidative injury, we directly measured the formation of lipid hydroperoxides in the presence and absence of EHT. This colorimetric assay reports on the redox reaction between iron and hydroperoxides. Our results indicate that EHT is able to significantly reduce the production of the ferric ion suggesting that it possesses antioxidant activity that may aid in maintaining membrane integrity. Next we studied EHT's potential to reinforce the structural integrity of neurons. Differentiated SH-SY5Y neuroblastoma cells were treated with lysophosphatidic acid (LPA) to induce neurite retraction. Upon treatment with EHT, an increase in neurite length was observed suggesting protection from LPA. Lastly, the neuroprotection of SH-SY5Y neuroblastoma cells against the chemical neurotoxin MPTP was evaluated. Treatments with EHT resulted in dose-dependent neuroprotection with an EC_{50} of approximately 100 nM.



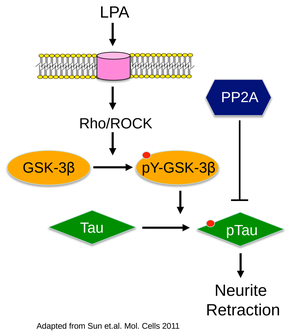
Eicosanoyl-5-hydroxytryptamide

Significance of PP2A and its Methylation State

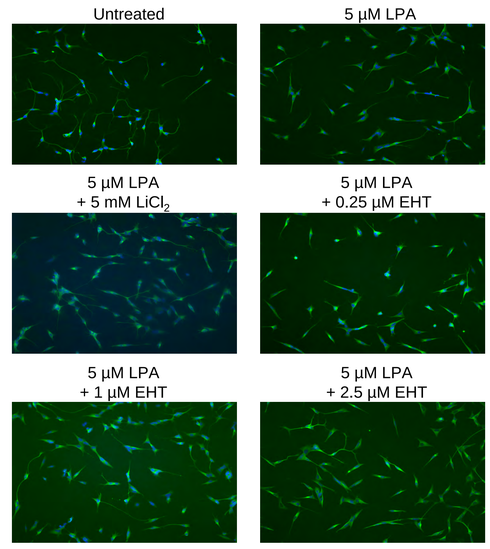


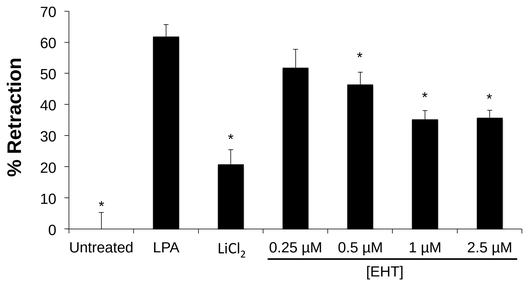
PP2A is a global cellular regulator that controls processes ranging from gene expression and development to morphogenesis and metabolism. The PP2A holoenzyme is formed by the association of its scaffolding A-subunit, catalytic C-subunit and one of a variety of different regulatory B-subunits. The S-adenosylmethionine-dependent methylesterification of the C-terminal Leu306 of the catalytic subunit of the PP2A-AC heterodimer is catalyzed by the leucine carboxyl methyl transferase (LCMT). This post-translational modification is reversed by a PP2A-specific methylesterase, PME. In the brain, PP2A has been estimated to be responsible for approximately 70% of the total phospho-Ser/Thr protein phosphatase activity with one of its main substrates being the microtubule associated protein Tau[8]. PP2A's requirement for S-adenosylmethionine closely links its methylation status to the one-carbon metabolism system within the cell[9]; a pathway that is altered in Parkinson's and Alzheimer's disease reflected in the observed decrease in PP2A activity[2-3] and methylation of its carboxy-terminal tail[4]. It therefor seemed likely that an agent that inhibits PME activity so as to maintain the methylation status of PP2A might provide a novel anti-neurodegenerative therapeutic. EHT was identified in a screen for coffee components with this activity and has been shown to have neuroprotective efficacy *in vivo* in rodent models for neurodegenerative disease[1,5-7]. Here we demonstrate EHT's protective effects in various *in vitro* models.

EHT Provides Protection from LPA induced Neurite Retraction



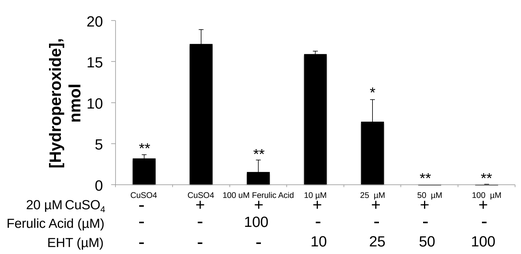
Adapted from Sun et.al. Mol. Cells 2011





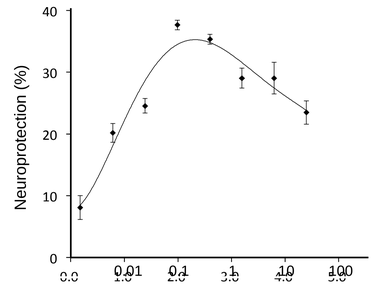
Alterations in microtubule dynamics caused by hyperphosphorylation of Tau can lead to pathological consequences such as those observed in Alzheimer's disease. Therefore we sought to determine if EHT could help maintain neurite stability. The neuroblastoma cell line, SH-SY5Y, was differentiated using 10 μM retinoic acid for 72 hours at 37ºC and 5% CO_2. Cells were pretreated with varying doses of EHT prior to treatment with lysophosphaditic acid (LPA). $LiCl_2$, an inhibitor of GSK-3β activation, was used as a positive control. Neuritic processes were visualized using an anti-β-tubulin primary antibody and Alexa Flour® 488 conjugated secondary antibody. Neuritic length was measured using the ImageJ plugin NeuronJ. Data are represented as the the mean ± SEM (n=9). *p < 0.001 compared with the LPA treatment group.

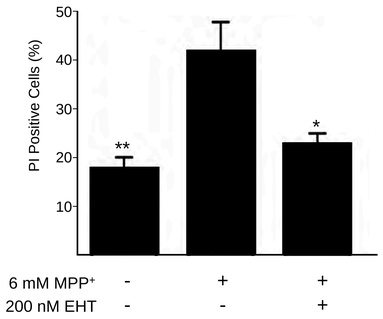
Antioxidant Properties of EHT Combat Lipid Peroxidation



The integrity of a cell is greatly influenced by oxidative damage of lipids. Therefore, we explored the antioxidant effects of EHT to prevent lipid peroxidation. Native low-density lipoprotein (LDL) was incubated overnight at 37°C in the presence of $CuSO_4$. Lipids were extracted into chloroform and assessed by the formation of a colorimetric complex between ferric ions and thiocyanate which was measured at an absorbance of 500 nm. Data are represented as the the mean ± SEM (n=4). *p < 0.01; **p < 0.001 compared with the +$CuSO_4$ treatment group.

EHT Protects the SH-SY5Y Cell Line From MPP^+ Cytotoxicity





1-methyl-4-phenylpyridinium or MPP^+, is the neurotoxic metabolite of MPTP that interferes with oxidative phosphorylation in mitochondria by inhibition of Complex I. We explored the ability of EHT to dose-dependently protect the SH-SY5Y neuroblastoma cell line from MPP^+ cytotoxicity. A 6 hour pretreatment with the indicated concentrations of EHT was followed by a 6 mM MPP^+ treatment for 24 hours (left) or 9 hours (right). Cellular viability was measured via lactate dehydrogenase activity in the media (left) or by propidium iodide staining (right). Data are represented as the the mean ± SEM (n=6). *p=0.0182 and **p=0.0053 compared with the MPP^+ treatment group.

Summary

- PP2A methylation system poses as a promising target for neurodegenerative disease.
- The coffee component, eicosanoyl-5-hydroxytryptamide (EHT), maintains PP2A methylation while reducing Tau hyper-phosphorylation.
- EHT is able to reduce the effects of LPA induced neurite retraction presumably by maintaining tau in a highly dephosphorylated state and thereby improving the structural integrity of differentiated SH-SY5Y cells.
- A reduction in lipid peroxidation was observed indicating an anti-oxidant activity for EHT which may aid in maintaining membrane integrity.
- EHT protects cells from the neurotoxin MPP^+ with maximal potency in the nanomolar range.
- The various neuroprotective properties of EHT provide further evidence for this coffee components role in overall brain health.

References

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