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The effect of secreted-amyloid precursor protein on NMDA receptors in cultured rat hippocampal slices. I Ballagh¹, D Ireland¹, B Mockett¹, K Bourne², W Tate², J Williams³, W Abraham¹. ¹Department of Psychology, ²Department of Biochemistry, ³Department of Anatomy and Structural Biology, University of Otago, Dunedin.

Alzheimer's disease has been linked to decreased levels of a neuroprotective protein, secreted amyloid precursor protein- α (sAPP α). sAPP α regulates memory-related synaptic plasticity in the hippocampus, a process dependent on the N-methyl-D-aspartate glutamate receptor (NMDAR). The present study investigates the effect of sAPP α exposure on NMDAR function in the rat hippocampus using an *in vitro* slice culture method.

Hippocampal slices from 7- to 12-day old rat pups were maintained in culture for 7-12 days. Recordings of isolated NMDAR-mediated excitatory postsynaptic currents (EPSCs) from the cell bodies of individual CA1 pyramidal neurons were obtained using standard patch clamping methods. Cells exposed to 1 nM sAPP α showed a significant reduction in the mean time course of the EPSC_{NMDA} evoked by a single stimulus (91.5 ± 9.3 ms, mean \pm SD, $n = 8$,) compared to untreated cells (123.0 ± 42.1 ms, $n = 11$, $P < 0.05$; unpaired *t*-test). Longer exposure to sAPP α led to greater depression of the time course ($P < 0.05$; linear regression). Exposure to 1 nM sAPP α increased the time course of the EPSC_{NMDA} evoked by a train of ten pulses (132.6 ± 25.6 ms, $n = 5$) compared to untreated cells (90.4 ± 8.0 ms, $n = 5$, $P < 0.02$; unpaired *t*-test).

Slice cultures obtained from 3 animals were grown in media containing 1 nM sAPP α for periods of 1, 6 and 24 h and processed to obtain postsynaptic density-enriched membrane fractions. Preliminary Western blot densitometric analysis showed increases in the concentration of the NR1 NMDAR subunit in the postsynaptic density at 6 h relative to untreated slice cultures in all 3 animals.

These results suggest that sAPP α can alter the functional characteristics of the NMDAR, perhaps through a change in the subunit concentration and/or composition. This could be a mechanism by which sAPP α affects synaptic plasticity.

Bioinformatically informed analysis of the gene transcription cascade following induction of long-term potentiation in the rat dentate gyrus. S Bisky¹, S Mason-Parker², W Abraham², J Williams¹. ¹- Department of Anatomy and Structural Biology, Otago School of Medical Sciences, ²-Department of Psychology, University of Otago, Dunedin.

This project aimed to use bioinformatics and quantitative realtime PCR (qPCR) to analyze a part of the genetic network responsible for maintaining long-term potentiation (LTP) in the rat dentate gyrus (DG). LTP is a well-established model for

memory, the persistence of which is dependent on activation of specific transcription factors (TFs) and their downstream response genes. Using bioinformatics, we aimed to find possible targets of some of these TFs based on binding sites in their promoter regions.

Known rat gene promoters obtained from databases were scanned for binding sites of four TFs involved in LTP (NF κ B, CREB, AP1 and EGR-1). Of these, 198 promoters contained sites for one or more of these factors, suggesting that these genes may be involved in LTP maintenance. To test the biological relevance of these results, two genes (coding for tissue plasminogen activator [tPA] and lipoprotein-receptor related protein [LRP]), whose promoters showed possible EGR-1 binding sites, was quantified following LTP.

Five rats were bilaterally implanted with electrodes to stimulate their perforant path and record excitatory postsynaptic potentials from granule cells of their DG. One hemisphere remained unstimulated as a within-animal control. After monitoring potentiation for 5h, the rats were killed and their DG removed, in order to isolate total RNA and perform reverse transcription. A SYBR-green based qPCR assay using gene-specific primers showed significant upregulation for LRP (average fold expression change: 1.84 ± 0.2 SEM, $P < 0.01$), as well as a smaller but significant change for tPA (1.5 ± 0.28 SEM, $P < 0.05$).

These results identify two possible downstream targets of EGR-1, and indicate that knowledge from database mining can be used to inform the search for parts of the LTP-related transcriptome puzzle.

Leptin does not act directly on gonadotrophin releasing hormone neurons to regulate fertility in rats. R Geddes, D Grattan, G Anderson. Centre for Neuroendocrinology and Department of Anatomy and Structural Biology, University of Otago, Dunedin.

The hormone leptin is produced by adipose tissue and has a central permissive role in regulating fertility. It provides the communication link between nutritional state and the fertility-controlling centres of the brain, such that low levels caused by undernutrition lead to infertility. The study aimed to determine, using immunohistochemistry, if this action of leptin is mediated directly through gonadotrophin releasing hormone (GnRH) neurons, the central drivers of fertility. As leptin receptors are difficult to detect, a downstream leptin-activated cell-signalling factor, phosphorylated signal transducer and activator of transcription 3 (pSTAT3) was used to identify leptin-activated cells.

Female Sprague-Dawley rats ($n = 16$) were food-restricted (60% of *ad libitum* intake) for 12 days to up-regulate leptin receptors and enhance leptin sensitivity. This feeding regime caused a 17% reduction in body weight compared to *ad libitum* fed rats. Recombinant mouse leptin (4 μ g) or vehicle was given to the animals intracerebroventricularly, 30 minutes prior to fixing the brain with 2% paraformaldehyde by cardiac perfusion. Coronal 35 μ m sections through the preoptic area of the hypothalamus were cut. Double-label immunohistochemistry was used to identify pSTAT3 immunoreactivity in GnRH neurons. GnRH neurons were identified using unenhanced diaminobenzidine tetrahydrochloride (DAB) and pSTAT3 identified using nickel-enhanced DAB, staining GnRH neurons brown and pSTAT3

black. Three sections evenly spaced throughout the medial septum and preoptic area were examined per rat, and all the GnRH neurons in these sections counted. Relatively little pSTAT3 staining was observed in vehicle-treated rats. In the leptin-treated group, pSTAT3 staining was present on numerous unidentified cells in the vicinity of the GnRH neurons, however none of the 246 neurons counted were co-localized with pSTAT3 staining.

These results provide compelling evidence that leptin does not act directly on GnRH neurons, but instead is likely to act through an indirect neuronal pathway to support fertility.

Discovery of Inhibitors of Fungal Plasma Membrane Proton Pump ATPase. R Keng, K. Niimi and B. Monk. Department of Oral Sciences, University of Otago, Dunedin.

Opportunistic fungal infections caused by *Candida* species have increased in recent years, particularly in immunocompromised individuals, and emergence of antifungal drug resistance has become a clinical problem. There are, however, only four classes of antifungal drugs available for treatment of systemic infections. The discovery of new classes of antifungal drugs is therefore urgent. The aim of this study was to discover broad-spectrum inhibitors of fungal plasma membrane proton pump ATPase (Pma1p), an essential enzyme for cell survival.

Thirty selected compounds, which have been identified as potent inhibitors of *C. albicans* Pma1p ATPase *in vitro* were obtained from Pfizer Inc. Each compound was tested against major pathogenic *Candida* species, *Cryptococcus neoformans* and a model yeast *Saccharomyces cerevisiae*.

One of the compounds (compound 10) gave broad-spectrum inhibition of the growth of pathogenic yeast species and *S. cerevisiae* (minimum inhibitory concentration [MIC] 6.25 - 12.5 μ M). Compound 10 inhibited the Pma1p ATPase activity of plasma membranes isolated from these species (IC₅₀ 0.2 - 9.4 μ M). It also acted as a pH sensitive chemosensitizer that made cells sensitive to a sub-MIC concentration of fluconazole (FLC), a widely used antifungal drug. It did not affect cell growth in the absence of FLC, indicating that compound 10 may also inhibit fungal ATP-binding-cassette (ABC) transporters.

Compound 10 was identified as a potent Pma1p inhibitor for all the fungal species tested. Further chemical modification will be required to improve its antifungal and chemosensitisation activities.

T-lymphocyte contribution in a model of cerebral ischaemia. K McKelvey, R Rahman, S Nair, J Ashton, I Appleton. Department of Pharmacology & Toxicology, Otago School of Medical Sciences, University of Otago, Dunedin.

Stroke is the third-leading cause of mortality and the leading cause of disability worldwide. Recent reviews have implicated inflammation as a major cause of the delayed progression of neural injury following stroke. Post stroke, a portion of the inflammatory response is induced and propagated by cytokines secreted by activated T-lymphocytes. Depending on the T-lymphocyte involved, the cytokines may enhance or dampen the inflammation, identifying them as a potential avenue for

therapeutic intervention. This study sought to quantify the contribution of T-lymphocytes in post-ischaemic neurodegeneration and delineate the immune cell response.

Male Sprague-Dawley rats (265 - 295 g) were utilised in a middle cerebral artery occlusion model of transient focal cerebral ischaemia. Animals (n = 4) were sacrificed at days 0 (non-intervention control), 3, 7 and 14 post-stroke induction. Acetone fixed cryostat sections were used in the immunohistochemical (IHC) labelling. Primary antibodies against CD3, CD4, CD8, interferon-gamma (IFN- γ) and interleukin (IL)-4 were visualised using horse-radish peroxidase (HRP) with chromagen 3'3' Diaminobenzidine tetrahydrochloride (DAB) or fluorochrome conjugated secondary antibodies. CD3 labelling, which identifies all T-lymphocytes, confirmed that there was a significant and sustained up-regulation in T-lymphocyte infiltration to the ischaemic area up to at least 14 days following stroke. CD4 and CD8 IHC labelling revealed that CD4 T-helper cells were the predominant infiltrate, as opposed to CD8 T-cytotoxic cells, in the ischaemic area.

Double immunofluorescence was used to differentiate between the two T-helper (Th) cell subsets, Th₁ and Th₂. Th₁ cells secrete the pro-inflammatory cytokine, IFN- γ leading to a cell-mediated response. Conversely, Th₂ cells secrete the anti-inflammatory cytokine, IL-4, producing a humoral response. Temporal profiling of the T-lymphocyte response illustrated that CD4/IFN- γ Th₁ cells were the major T-helper cell present in the infarct.

In conclusion these results demonstrate that the immune component of the prolonged neurodegeneration following stroke is a CD4 Th₁ cell-mediated response.