Research Report to the Waikato Medical Research Foundation

Project title: Development of Mimetic peptides for targeted modulation of gap junctions in the cerebral cortex – potential for control of seizures

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Phase 1 – Design of Mimetic peptides to specifically block gap junction channels

We aimed to design and test novel peptides that give blockade of specific subtypes of cell-to-cell connections called gap junctions that we believe to be important in seizure formation. To identify good target regions unique to individual subtypes of gap junction protein, we first performed some simple alignments of sequences for all connexins known to be expressed in the brain, including connexins -32, -36,- 43 using the software CLUSTALX. This allowed identification of regions that were: a) in an extracellular loop region crucial to formation of functional gap junctions (which we aim to disrupt during their formation) and b) unique to particular gap junction proteins of interest, such as connexin36. We were able to identify a number of mimetic peptides that we believe are likely to give moderately stringent and highly specific blockade of Cx36 gap junction channels (table 1: numbers 3, 5, 7 and 9). Alongside these peptides we also planned to test peptides that other groups had found to be effective blockers of connexin43 based gap junction channels (table 1: number 1). We used a scrambled sequence matched control for each targeting peptide (indicated as: "sequence name"_scrambled) in table 1.

	Peptide name	A.A. sequence	Mole. Weight..
1	**Gap27**	**SRPTEKTIFII**	MW 1304.57
2	Gap27_scrambled	FKRIITPEITS	MW 1304.57
3	Gap27_Cx36	SRPTEKTVFLV	MW 1276.51
4	Gap27_Cx36_scrambled	TRSKTVEVFLP	MW 1276.51
5	Peptide5_Cx36	VECYVSRPTEKT	MW 1411.62
6	Peptide5_Cx36_scrambled	TVKVYRCTPEES	MW 1411.62
7	Cx36_specific	GLYECNRYPCIK	MW 1458.74
8	Cx36_specific_scrambled	EPYYGKCCRLIN	MW 1458.74
9	Gap26_Cx36	ACYDRAFPISHIR	MW 1548.81
10	Gap26_Cx36_scrambled	PRYIHRCDFAISA	MW 1548.81

Phase 2- Developing techniques to ensure long term slice viability

The success of this study depends upon two important factors relating to brain slice viability for extended periods of time:

1) Firstly, because mimetic peptides are likely to exert their effects only after quite long periods of treatment, it was important to first establish a system for maintenance of neocortical brain slices for ≥10 hours in a viable state and that these slices had the capability to show seizure like event (SLE) activity. Around 10 hours is generally considered a limit for mouse neocortical slice survival. As a prerequisite it was therefore necessary to establish whether, and under what conditions, slices would remain viable for this length of time. Several set-ups were trialed. It was established that the optimum conditions were for slices to be maintained in 10mL of no-magnesium artificial cerebrospinal fluid (aCSF) and continuously bubbled with carbogen (95% oxygen, 5% carbon dioxide) (Figure 1). The aCSF did not need to be circulated as is commonly done in neocortical slice studies. Under these conditions, 60-70% of slices continued to exhibit SLE activity in no-magnesium aCSF 14-16 hours after tissue slicing. Obtaining this level of ongoing SLE activity was dependent upon the age of the animals, with 4-5 week old mice being optimum. Younger and older animals yielded less robust activity.

2) Secondly, being able to deliver the mimetic peptides (the experimental test compounds) to the tissue via the aCSF is crucial to the success of the experiment. We discovered that the peptides, while soluble in distilled water, did not remain in solution when added to the aCSF. We trialed two solubilizing agents, acetic acid and dimethyl sulfoxide (DMSO). Acetic acid at 0.2% successfully dissolved the mimetic peptides but was toxic to the slices. DMSO at 1% was non toxic to the slices and will be trialed in further experiments as a mimetic peptide vehicle.

Phase 3 – Measuring effects of peptides on seizure-like events

We are now testing the effect of the peptide sequences on seizure-like events (SLEs) in cortical brain slices to test our hypothesis that specific blockade of connexin36 gap junctions will have an effect on the SLEs (potential either pro- or anti-seizure) . We anticipate that we should be able to publish our preliminary findings by the first quarter of 2012.

Because we were able to find salary support from other sources, there are sufficient funds in the grant allocation for consumables and animal housing costs to carry on this project well into 2012.

Breakdown of expenditure:	**$NZD**
Mimetic peptides x10 (synthezised by Genscript, USA)	**$3021**
Peristatic pump	**$2836**
Animal maintenance costs	**$5000**
Lab consumables	**$3259**
Gases for electrophysiology	**$780**
Total spend to date	**$14896**

Figure 1. Equipment used in measurement of seizure-like events in cortical tissue within rodent brain slices with control or gap junction targeting peptide. a) Stereomicroscope and recording apparatus and b) detail of recording apparatus.

a) **b)**