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The effect of Alzheimer's disease—associated β-amyloid peptide on voltage-dependant currents in a human-derived cell line

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Alzheimer's is an extremely debilitating disease characterised by the progressive degeneration of neurones from regions of the brain predominantly involved in memory storage. This leads to the characteristic symptoms of progressive memory loss and a gradual decline in cognitive ability. Senile plaques form one of the main pathological structures associated with the brains of patients with Alzheimer's Disease (AD). They consist predominantly of an aggregated from of a peptide known as β-amyloid (Aβ). These plaques are often co-localised with populations of degenerating neurones and one popular hypothesis is that they may be responsible for neuronal death. Work on a variety of animal species has led to a model whereby Aβ is believed to be excitotoxic leading to uncontrolled increases in intracellular Ca^{2+} (Mattson, 1993). The majority of these studies have been carried out using animal models and therefore their significance to a solely human disease is unclear. We have used a cell line (NT2) derived from a human teratocarcinoma that when differentiated with retinoic acid takes on a neuronal phenotype (hNT). This study examines the effects of the Aβ(1–40) peptide on the properties of the voltage-gated membrane currents present in these cells.

Single differentiated hNT cells were voltage clamped in the whole cell configuration using a modification of the patch clamp technique originally described by Sackmann and Neher. Whole cell membrane currents were isolated either pharmacologically, or by the use of voltage clamp protocols that made use of the differing voltage sensitivities of the component currents.

hNT cells contained both voltage-gated Na^+ and K^+ currents. The Na^+ current activated at membrane potentials between –50 and –40mV increased in amplitude to peak between –30 and –20mV and then declined at more positive potentials before reversing around +40mV. The current was fast to activate and inactivated completely during the 200ms voltage step. The amplitude of this current was reduced significantly in a saline in which the Na^+ concentration was reduced by 90%, ($p<0.01$) indicating that Na+ ions were the main charge carrier through these channels. All these properties characterise this current as a typical voltage-gated Na^+ current. The voltage-gated K^+ current activated between –40mV and –30mV. The amplitude of the current increased as the potential to which the cell was stepped became more positive. The current was fast to activate and showed relatively little inactivation during the 200ms voltage step. The current was more sensitive to 4-aminopyridine than to tetraethylammonium ions (50% block 5mM and 150mM, respectively). These properties tend to characterise this current as a member of the delayed rectifier K^+ current family.

Application of the aggregated Aβ (50μM) caused a significant but irreversible reduction in the amplitude of the K^+ current (52.0±8.87%; mean ± SE, n=6; $p<0.01$). Aggregated Aβ had no effect on the amplitude of the Na^+ current. Suprisingly, applications of the unaggregated ("non-toxic") form of the peptide also reduced the amplitude of the K^+ currents (11.6±1.7%; mean ± SE; n=6; $p<0.05$). The were no effects of the unaggregated peptide on Na+ current amplitude.

These data are the first to describe the effects of Aβ(1–40) on Na^+ and K^+ currents in a human neuronal cell line. The ability of the aggregated ("toxic") peptide to block K^+ currents is consistent with the hypothesis that the peptide is excitotoxic and has the ability to disrupt intracellular Ca^{2+} homeostasis. The results also confirm the work of Good (1996) who demonstrated similar actions of this form of the peptide on K^+ currents in rat hippocampal neurones. The results obtained with the unaggregated form suggest that this form of the peptide is also active on K^+ channels, however, at present the significance of this result is unclear.

Mattson et al. (1993) TINS. 16: 409-414.
Good, T.A. (1996) Biophysical Journal 70: 296-304.