P007 Proteolytic processing of proneurotrophins and their effects on vascular smooth muscle cell behaviour.

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The neurotrophins and their receptors are expressed on vascular smooth muscle cells (VSMC) and may play a role in the cardiovascular system in the pathogenesis of disease states including atherosclerosis. The aim of this study is to test the hypothesis that plasmin and other membrane- associated serine proteases are regulators of VSMC survival and apoptosis through extracellular processing of proNGF and proBDNF.

A Drosophila expression system has been developed for wild type and furin-cleavage resistant NGF and BDNF. The furin-cleavage resistant mutants have been created through mutagenesis of the primary consensus sequence for furin (RXRR→RXAA) to allow high level expression of proneurotrophins. The processing of the purified proproteins has been investigated in activation assays with serine proteases including plasmin, thrombin and hepsin. Preliminary time course experiments show that purified proBDNF and proNGF are activated at very low plasmin concentrations over a short time period. The specific protease cleavage sites will be identified with mass spectrometry.

Using real-time quantitative PCR the effects of purified pro- and mature neurotrophins on VSMCs gene expression have also been investigated. Our data show that a number of proteases and related genes have altered expression in response to neurotrophin treatment. The effect of neurotrophins on VSMC behaviour will be investigated in relation to survival, migration and apoptosis.