51 Haem oxygenase/nitric oxide synthase interaction: a role in neurodegeneration?


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Excessive generation of reactive nitrogen species (RNS) has been implicated in the pathogenesis of a number of neurodegenerative, inflammatory diseases, e.g. Multiple Sclerosis (MS). It has been shown that in vitro exposure of astrocytes to interferon gamma (IFNγ) + lipopolysaccharide (LPS) results in induction of nitric oxide synthase (NOS), increased generation of RNS and a corresponding inhibition of the mitochondrial respiratory chain. Inducers of NOS also cause induction of haem oxygenase (HO) protein and activity. We have shown that IFNγ + LPS or hemin induce HO-1 expression and activity in primary rat astrocytes. Hemin-induced HO-1 expression and activity is prevented by the HO inhibitor tin protoporphyrin IX and additional experiments suggest inhibition of HO-1 expression by a specific NOS inhibitor. Our findings confirm previous reports that interactions between the HO/NOS systems occur in astrocytes. We postulate that such interactions are likely to play a pivotal role in the development of neurodegenerative diseases such as MS. Furthermore, glad over-expression of HO-1 in this disease state has recently been reported.

52 An investigation in changes in gene regulation and subsequent signal transduction activation after ischemic stroke

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We investigated the status of potent angiogenic and apoptotic factors in human post mortem samples from patients who died following acute ischemia and rats after middle cerebral artery occlusion. cDNA multi-array technology was used to screen rats for changes in gene expression at time points 0-7 day after induction of ischemia. Initial results after 1-12 hours showed an increase in a significant number of relevant proteins, which are under investigation, including TNFα, PDGFβ, TGFβ and II-1B.

53 Controlled release of cytochrome c from functional brain mitochondria; implications for apoptotic mechanisms in neurodegenerative disease.

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Release of cytochrome c from the inter-membrane space of mitochondria to the cytosol is a process understood to initiate apoptosis, which itself underlies much of the cell loss during neurodegenerative disease. The precise molecular mechanism by which cytochrome c release occurs is as yet unclear, particular interest having arisen over whether it necessarily correlates with loss of mitochondrial function. In this study, the function of isolated non-synaptic rat brain mitochondria has been titrated using the specific complex I inhibitor rotenone, since mitochondrial respiratory chain complex deficiency is a feature of several neurodegenerative conditions. The progressive impairment of mitochondrial function correlated with increasing cytochrome c release. Despite showing some sensitivity to cyclosporin A, an inhibitor of the mitochondrial permeability transition, this release was unassociated with any mitochondrial swelling or cyclosporin-sensitive dissipation of membrane potential. We conclude that cytochrome c is released from mitochondria in a controlled, proportional to the extent of insult. The mitochondria can meanwhile remain functionally and morphologically intact. Our data have important implications for brain recovery during neurodegenerative disease. They also suggest a more subtle role for cytochrome c release than commonly perceived, perhaps implicating an intracellular signalling mechanism.

54 Molecular and cellular characterisation of the Prion-like protein Doppel

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The prion protein (PrP) is the causative agent of the transmissible spongiform encephalopathies, including Creutzfeldt-Jakob disease (CJD) in humans. Doppel (Dpl) was recently discovered as the first PrP related protein. Dpl is encoded by the novel gene locus Prnd, located on the same chromosome as the Prnp gene that encodes PrP. When ectopically expressed in the neurons of PrP deficient mice, Dpl was seen to cause ataxia due to Purkinje cell degeneration. There is also evidence to suggest that Dpl and PrP may interact, human Dpl has been stably expressed in the SH-SY5Y neuroblastoma cell line which lacks endogenous PrP. Dpl expressed in these cells is glycosyl-phosphatidylinositol (GPI) anchored and N-glycosylated. Sucrose density gradient centrifugation in the presence of Triton X-102 has revealed that Dpl is present in cholesterol-rich lipid rafts. The effect of Dpl on cellular copper homeostasis and the resistance of the cells to oxidative stress, two processes in which PrP have been implicated, is being investigated.