Report on Apoptosis — The Role of the Mitochondria in Cell Death

The word 'Apoptosis' in its biological sense was first coined in 1974, but the word derives from the Greek for 'falling leaves'; today it is still used in Greek as a term for baldness. With that in mind, Professor Tim Greenamyre opened the SMR symposium on this subject on 13 July 2000, with a plenary talk on the role of apoptosis in a number of neurological diseases.

Huntington's disease (HD) is a rare but devastating disease of familial origin caused by a protein called *huntintin*, of unknown function. The gene responsible for the disease is an autosomal dominant mutation in 5 to 10 people per 100,000. Huntingdon's disease is uniformly fatal 15 years after onset, which is normally in the 4^{th} or 5^{th} decade of life.

Pharmacologists have attempted to model the disease with neurotoxic injections, and there are similarities to kainate- or NMDA-induced neutotoxicity. Both of these systems are of the glutaminergic type of receptor, but the defects lie in the mitochondria rather than in the glutaminergic receptor system. Mitochondrial depolarisation is easier in HD patients, such that normal concentrations of glutamate cause nerve cell excitation. Mitochondrial inhibitors such as malonate or 3-nitropropionic acid act in synergy with glutamate agonists like NMDA to cause cell death. Calcium homoeostasis is disrupted and HD mitochondria are less able to continuously repolarise after ionophore treatment that normal mitochondria.

Parkinson's disease is another with apoptotic connections, but this time with both familial and sporadic origins. It is estimated to affect over 1% of over 55s. PD involves neurodegeneration of the dopaminergic neurons in the substantia nigra that participate in movement. One of the central questions is why these neurons in particular are affected. One theory is that the biochemistry of dopamine production is particularly prone to the production of free radicals and this places dopaminergic neurons under greater stress than other nerve cell types. It has been postulated that the substantia nigra is more exposed to neuroexcitation (over other dopaminergic areas) because of the glutaminergic input from the sub-thalamic nucleus. Pharmacological models have involved the intracerebral injection of the neurotoxin 6-OHDA into the substantia nigra. The slow degeneration that this causes can be inhibited by up to 50% by a glutaminergic antagonist like MK-801.

Professor Andrew Halestrup moved the discussion on from the head to the heart, with a talk concerning the importance of apoptosis in myocardial ischemia. It is noteworthy that in heart attack patients, there is often a necrotic core around the area affected by the thrombosis, which is surrounded by an apoptotic penumbra. Whether a cell lives or dies following damage is determined by the level of ATP that can be provided by the mitochondrion. The process of apoptosis is thought to involve the opening of a pore in the mitochondrion that makes it leaky to molecules less than 1500 Daltons molecular weight. The pore opening can be triggered by calcium, and sensitised by low concentrations of adenine nucleotides and oxidative stress. The proposed mechanism is that cyclophilin D (a unique mitochondrial cyclophilin) binds to adenine nucleotide transferase (the pore protein) and thereby enables the calcium triggering. ATP can inhibit the pore opening, but of course levels of ATP are lowered under conditions of oxidative stress. Cyclophilin plays a central part in this

mechanism, but its ability to bind to the adenine nucleotide translocator is impaired in the presence of cyclosporin A, to which it binds.

In reperfusion injury, cells damaged in heart attacks or in stroke deteriorate further once the thrombotic block is removed, and eventually die. This process is apoptotic rather than necrotic. Cyclosporin A (CsA) protects against ischemic damage in this respect, but the therapeutic window is very narrow. CsA also protects in the confocal ischemia model of stroke. The effect can also be modelled *in vitro*, with radiolabelled deoxyglucose as a tracer for pore opening in ischemic cells. A number of drugs can be examined in this model for their effects on mitochondrial pore opening. Propofol is an effective agent in this respect, because it acts as a radical scavenger and inhibits reperfusion injury, it protects in functional parameters such as cardiac work. Pyruvate is another: it works in three ways, to lower pH, as a free radical scavenger and to increase ATP levels. However, interestingly, although pyruvate is very effective in retaining function, it dose not prevent the mitochondrial pore from opening. The full story behind the pore and apoptosis is still to be unravelled.

Professor Ian Reynolds finished the morning session with a lively presentation on the measurement of mitochondrial function. His work centred on striatal neuron cultures, which could be labelled with various dyes to measure mitochondrial potential and oxidative stress. Dichlorofluorescein is an oxidation-sensitive dye, which can be used to establish that within 2 minutes of glutamate exposure, these neurons start to produce free radicals. However, the ultimate future of the cell is not established until up to 24 h after glutamate exposure. IN the interim, calcium entry into the mitochondria is a critical determinant of survival, particularly in its role as a driver of free radical production. Professor Reynolds believed that the permeability transition pore referred to by the previous speaker is not the only mechanism. The levels of calcium in the mitochondria can be calculated to be surprisingly high: the mitochondrion is roughly 5% of cell volume, but contains a substantial proportion of cellular calcium.

Using a mitochondrial voltage sensitive dye (JC-1), Reynolds showed that even at ‘rest’, mitochondria in neurons and in astrocytes are continuously depolarising and repolarising. ATP inhibition dramatically inhibits this process, and glutamate shows a substantial effect on the mitochondrial potential in this system; however, it has proved to be more difficult to show the effect of drugs like CsA and tetrodotoxin.