

The current projects in our laboratory are designed to identify the processes underlying the neuronal cell death pathways involved in neurodegenerative diseases such as Parkinson and Alzheimer diseases. Our specific goal is to define the signal transduction pathways leading to apoptosis or survival in neurodegenerative process, since we believe that elucidating pharmaceutical targets that alter the cellular balance in favour of apoptosis or survival would provide effective treatments for a variety of neuropathological conditions. A multidisciplinary approach that integrates cellular cultures and isolated mitochondrial preparation is used for this propose.

Multidisciplinary approach

- **Cell cultures:**
 - Primary neuronal cultures, hippocampus, cortex, cerebellum
 - Cell line cultures
- **Cell viability assays:**
 - MTT, DFCA, LDH, Hoechst
- **Microfluorimetry:**
 - Levels of second messengers: Calcium, superoxide, peroxide, NO.
 - Mitochondrial funtion: in situ PTP formation, collapse transmembranal portential, morphology
- **RNA and protein:**
 - RT-PCR, WB,
 - Immunofluorescence
 - Protein overexpression
- **Isolated Mitochondria:**
 - Swelling
 - Transmembranal potential
 - Oxygen consume
 - Calcium uptake
- **Enzymatic activity:**
 - Caspase
 - Calpain
 - Cytrate synthase
 - ECT complex
 - Nitric oxide synthase

Two main projects are presently running in our lab.

Project 1. Parkinson's disease is characterized by impaired motor control, including tremor, rigidity and slowness of movement. These disturbances are due to the loss of dopaminergic neurons. One of the current major hypotheses of this neuron loss is that it is due to programmed cell death (also known as apoptosis), a form of death in which genes intrinsic to the cell bring about its own destruction. We have used cellular cultures to study what apoptotical pathways are involved in 6-hydroxydopamine-induced cell death, a well established

model to study Parkinson's disease. The working hypothesis is that 6-hydroxidopamine (6-OHDA) results in the auto-oxidation and the release of pro-apoptotic factors from the mitochondria. We have shown that apoptosis can be induced by 6-OHDA in different cell types including, bovine chromaffin cells, cerebellar granular cells and the neuroblastoma cell line SH-SY5Y. We have focused our research on examining how the mitochondria become involved in these processes. We have a particular interest in identifying upstream mediators which may serve as therapeutic targets. Thus, mitochondrial release of cytochrome c in models of apoptosis is a critical event for caspase activation in many cells. Interestingly, our results indicate that intracellular second messenger, calcium and reactive oxygen species may regulate mitochondrial function in different ways. We found that whereas the calcium is sufficient to trigger cytochrome c release by inducing mitochondrial collapse in isolated rat brain mitochondria, reactive oxygen species induce swelling and cytochrome c release in the absence of transmembrane depolarization.

Project 2. This is directed toward understanding the cellular mechanism activated by the DNA damage response during cell injury. The p53 tumor suppressor gene is activated in response to DNA damage and mediates both cell cycle arrest and apoptosis. Previous research by this team has demonstrated that the pro-apoptotic function of p53 is largely mediated via the transcriptional upregulation of the BH3 only protein PUMA. We have demonstrated that depolarisation- and Ca^{2+} induced apoptosis also induce the transcriptional activation of the p53 target gene PUMA. The goal of this project is to investigate the mechanisms of p53 and PUMA activation during Ca^{2+} induced cell injury in excitable cells. In particular, the project addresses the question whether an increased Ca^{2+} -induced free radical formation triggers a DNA damage response, and whether p53 activation and PUMA induction are required for depolarisation- and Ca^{2+} -induced apoptosis.

Applications to new drug development:

Our experimental models allow to investigate the action mechanisms of plausible neuroprotective drugs such as minocycline. Minocycline, a semisynthetic derivative of tetracycline, has been shown to display beneficial activity in neuroprotective models. We are investigating the effects of minocycline on mitochondria, and our findings indicate that minocycline fails to block superoxide-induced mitochondrial swelling but is effective in blocking mitochondrial swelling induced by calcium. Therefore, our data clarify the mechanisms by which, minocycline fails to protect SH-SY5Y cell cultures against reactive oxygen species-mediated cell death, including malonate and 6-hydroxydopamine treatments, but is effective against staurosporine-induced cytotoxicity. In conclusion, with the type of strategy used to analyze minocycline we may contribute to develop novel treatments for several neuropathological diseases.