

Overall research theme:

**Integrative studies of cerebrovascular function and cerebral metabolism: Pathophysiological mechanisms and pharmacological approaches**

Latest update:

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Characteristics of the research group:

Areas of interest include hypoxia, cerebral metabolism, cerebral and cerebrovascular inflammatory mechanisms, and cerebral water channels (aquaporins). The work of the Research Unit ranges from basic experimental research in animals or healthy human individuals to full-scale controlled studies in patients. Activities is based on a close collaboration with other groups within the University of Copenhagen and Rigshospitalet, i.e. the Department of Neurosurgery, The Neurobiology Research Unit, the Department of Pharmacology, and the Neuropsychiatry Research Unit. In addition, the Research Unit participates in international studies on high altitude physiology.

Supported by internal and external grants, the Research Unit has been able to implement modern scientific tools into the specific projects. These include intracerebral microdialysis for elaboration of metabolism and pharmacokinetics, direct measurements of cerebral oxygen tension, myographic measurements in isolated arteries, and advanced bio-techniques for measurements of tissue expression of specific proteins and their mRNA.

Currently, the main interest is focused on the potential use of human, recombinant erythropoietin (EPO) as an antiinflammatory and neuroprotective drug. Recently, we demonstrated that a single subcutaneous bolus of EPO prevented the loss of cerebral autoregulation after experimental subarachnoid haemorrhage (SAH) in rats. Subsequent studies in isolated cerebral arteries showed that EPO normalized endothelium dependent vasodilation and contraction responses disrupted by SAH. Ongoing experimental studies in our laboratory aim to evaluate the effect of EPO on nitric oxide synthase isozymes in the brain and in cerebral vessels in animals with neuronal damage following SAH or hypoxic exposure. Within the frame of a Ph.D.-project, the Research Unit has now initiated clinical studies testing the neuroprotective potential of EPO in SAH patients. A similar protocol concerning head trauma patients has recently been approved by the regional ethical committee. The EPO studies have placed the Research Unit in a frontline position regarding this new evidence, and the ongoing clinical study remains as one of the first initiated thus far.

The EPO project includes collaboration with research groups within the Heart Centre concerning the effects of EPO in an animal model of myocardial ischaemia, and the effects of EPO on the inflammatory response following coronary bypass-surgery. Furthermore, an ongoing study in our laboratory is testing whether EPO in normal subjects inhibits the inflammatory response and muscle cell DNA damage following excessive, supramaximal exercise. During the last six month, the Research Unit has joined an international EPO research group, led by professor *Hannelore Ehrenreich* at the Max Planck Institute of Experimental Medicine, Göttingen, Germany.

Based on a co-work with the Department of Pharmacology, University of Copenhagen, preliminary work has been initiated for a Ph.D.-project investigating the role of specific cerebral water channels (aquaporins) in cerebral edema formation. Thus far, the laboratory have successfully set up immunoblotting methods for measurement of protein expressions of aquaporin subtype 4 which predominates in the brain. A hypoxic animal model has been developed for the induction of cerebral

edema. Currently, immunoblotting techniques using antibodies against aquaporin-1 and aquaporin-9 are evaluated. By the development of suitable animal models, the Ph.D.-project will aim to elucidate the function and regulation of cerebral aquaporins in relation to other important systems that are activated during edema formation, i.e. vascular endothelial growth factor and arginine-vasopressin.

New evidence indicate that the apolipoprotein E (ApoE) genotype may influence the outcome following head trauma, stroke or intracerebral haemorrhage: Patients with a ApoE4 genotype present with a higher mortality than patients with the ApoE2 and ApoE3 genotypes. In collaboration with the Neuropsychiatry Research Unit and the Neurobiology Research Unit, the outlines for a Ph.D.-project investigating the impact of ApoE genotype for the development of secondary brain damage is now being elaborated. A pilot study including patients admitted to the neurosurgical intensive care unit has been started to test the hypothesis that the presence of a ApoE4 allele is associated with a higher production of proinflammatory cytokines. Future studies are planned to also involve measurements of cerebral metabolism and lipid peroxidation by the use of intracerebral microdialysis.

#### *Research facilities*

The Research Unit is housed in locations that formerly constituted the Neurochemical Research Unit at the Department of Psychiatry. The laboratory facilities have been modernized to adequately meet the requirements for the conductance of experimental studies in human subjects. Office facilities offers deskplace and PC for at least five staff members. The laboratory facilities are shared with the Neuromuscular Research Unit, led by associate professor *John Vissing*, Department of Neurology. The location benefits from its proximity to the Neuropsychiatry Research Unit and the admittance to the advanced and well-equipped analytical laboratory of this unit. Internal and external funding allows the affiliation of a full-time technician.

The Research Unit conducts its experimental animal research within the facilities of the Neuropsychiatry Research Unit at *Juliane Maries Vej*, Rigshospitalet, and at facilities within the Department of Pharmacology at the Panum Institute. Immunoblotting methods are for the time being performed at the Department of Pharmacology, whereas biochemical measurements including PCR and ApoE genotyping are performed in collaboration with the Neuropsychiatry Research Unit.

A donation from the Toyota Foundation has allowed the purchase of a fully equipped device for bedside microdialysis (CMA Microdialysis Analyser) which is currently used in clinical studies at the intensive care unit. By other grants, the Research Unit has set up a compatible system for intracerebral microdialysis in rodents. Both internal and external fundings have allowed the purchase of basic laboratory equipment such as centrifuges, high-performance freezer, and a Radiometer ABL blood gas analyser including an OSM3. Internal funding has allowed the buying of a LICOX system for direct intracerebral measurements of oxygen tension in intensive care patients. For the use in specific protocols, the Research Unit has developed a hypoxic animal model, allowing the conductance of intracerebral microdialysis during hypoxic exposure in conscious animals.

#### *Running projects: Titles and abstracts:*

**Neuroprotective effects of human recombinant erythropoietin (EPO) in patients with acute subarachnoid haemorrhage (SAH).** In recent years, several independent research groups have demonstrated a remarkable neuroprotective and anti-inflammatory potential of EPO. Recent studies have established that neurons and astrocytes are capable of EPO production and that neurons, glial cells and brain capillary endothelial cells express specific EPO receptors. *In vivo* animal studies have shown that both intracerebroventricular and systemic administration of EPO protects against neuronal damage in models of stroke. Moreover, EPO improves survival in rodents after focal brain ischaemia and SAH. SAH secondary to rupture of an intracranial aneurysm is a life-threatening condition that often strikes young and middle-aged adults. SAH results in death or severe disability in 50 – 70 % of the patients and is the cause of up to ¼ of all cases of strokes. SAH often lead to vasomotor instability resulting in cerebral vasospasms and impaired autoregulation of cerebral blood flow. Cerebrovascular perturbations contribute

significantly to the development of delayed cerebral ischaemia which is the major cause of a poor clinical outcome in patients surviving the initial bleeding. Studies in our laboratory have demonstrated that a single subcutaneous bolus of recombinant, human EPO (400 IU/kg) normalizes cerebral blood flow autoregulation after experimental SAH in rats. Thus, early activation of endothelial EPO receptors may represent a potential therapeutic strategy in the treatment of cerebrovascular perturbations after SAH. This randomized, double-blind and placebo-controlled study is designed to evaluate the efficacy/efficiency of early administration of EPO as compared to placebo in the acute treatment of SAH. The hypothesis is that EPO will reduce mortality and morbidity. The primary endpoints are mortality and functional recovery. Secondary endpoints include the incidence of cerebral vasospasms and cerebral ischaemia. Measurements include assessment of cerebral metabolism and markers of inflammation by intracerebral microdialysis, intracerebral oxygen tension by a LICOX device, evaluation of cerebral blood flow autoregulation, and determination of ischaemic damage by diffusion- and perfusion-weighted MR-scans. The studies are conducted within a Ph.D.-project granted by the University of Copenhagen as a 3-year fellowship to Dr. *Jacob Springborg* (1.4.2002 – 31.3.2005).

**Nitric oxide synthase (NOS) isozymes and neuroprotective effects of erythropoietin (EPO) after experimental subarachnoid haemorrhage (SAH) in rats.** By the use of well established animal models the project aims to 1) characterize the expression, location, and activity of cerebral and cerebrovascular NOS isozymes after SAH, and to study the effect of iNOS/nNOS induction on the expression and activity of eNOS; 2) to test the effect of recombinant EPO on NOS isozymes; and 3) to evaluate the significance of the endogenous EPO/EPO-receptor system in relation to SAH induced changes in NOS isozymes and the development of cerebral vasospasms and ischaemia. In separate studies, the following hypotheses are tested: 1) SAH-induced cerebral ischaemia is aggravated by unspecific inhibition of NOS isozymes; 2) specific inhibition of iNOS and nNOS abolish SAH induced ischaemia and the associated downregulation of eNOS; 3) recombinant EPO prevents upregulation of iNOS and nNOS and also the downregulation of eNOS; 4) specific blockade of EPO receptors aggravates SAH induced cerebral ischaemia and is associated with a potentiated inhibition of the eNOS system. The project is supported by grants from the Danish Medical Research Council (kr. 500.000) and the Novo Nordic Foundation (kr. 150.000). Internal funding has allowed the affiliation of a scholarship (*Thomas Ersted*, 1.2.2002 – 31.1.2003).

**Expression of specific cerebral water channels (aquaporins) in cerebral edema and the interaction with vasopressin (AVP) and vascular endothelial growth factor (VEGF).** The purpose of the project is to study receptor mediated effects of VEGF and AVP on the expression of the cerebral aquaporin subtypes 1, 4 and 9 during development of cerebral edema in rats. Primarily, two models are used for the induction of cerebral edema: a hypoxic model (6 % oxygen for 48 hours), and intracerebral infusion of VEGF via chronically inserted osmotic minipumps. In separate series, the receptor mediated effects of VEGF and AVP are studied by co-infusion of specific agonists and antagonists (the VEGF-receptor-tyrosinekinaseinhibitor Tki, AVP V<sub>1</sub>-receptor antagonist, and AVP V<sub>1</sub>-receptor agonist). The project is intended to constitute a Ph.D.-fellowship held by Dr. *Tine Bernhardtzen*. The preliminary studies are supported by grants from the Sigurd Abrahamson Memorial Foundation.

**Significance of apolipoprotein E (ApoE) genotype for development of secondary brain damage following head trauma or intracerebral haemorrhage.** Patients with a ApoE4 allele present with a poor outcome compared with patients not having the ApoE4 allele but the pathophysiological mechanisms remain unknown. This project examines the relation of different ApoE genotypes (ApoE2, ApoE3, ApoE4) to known clinical and biochemical markers of inflammation and cerebral ischaemia in neurosurgical intensive care patients. A pilot study has been started to test the hypothesis that the presence of a ApoE4 allele is associated with a higher production of proinflammatory cytokines (IL-1 $\beta$ , IL-6, TNF- $\alpha$ , NF- $\kappa$ B) and their mRNA. Future studies are planned to also involve measurements of cerebral metabolism and lipid peroxidation by the use of intracerebral microdialysis. The project is intended to constitute a Ph.D.-fellowship held by Dr. *Jørgen Degn*. The preliminary studies are supported by grants from the Sigurd Abrahamson Memorial Foundation.

**Effect of human recombinant erythropoietin (EPO) on the inflammatory response to supramaximal exercise in humans.** EPO has been demonstrated to possess marked antiinflammatory effects in animals models of oxidative stress. This controlled, double-blind, randomized, cross-over study test the hypothesis that EPO in normal subjects inhibits the inflammatory response and muscle cell DNA damage following excessive, supramaximal exercise. The methods include muscle biopsies for measurements of tissue expression of iNOS, haemoxigenase, PARP, DNA single strand breaks, and oxidative DNA damage. The same measurements are obtained in isolated lymphocytes and leukocytes, in which mRNA for cytokines are also determined. The study is conducted by Dr. *Louise Vennegaard Kalialis*.

In collaboration with other research groups, the Research Unit is currently engaged in the following studies performed in other units:

1. Effect of EPO on myocardial ischaemia in rats (The Heart Centre, associate professor *Ole Amtorp*).
2. Effect of EPO on inflammatory response to coronary bypass surgery (The Heart Centre, consultant *Troels Dirch Poulsen*, and professor *Daniel Steinbrückel*).

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