Nitric oxide in focal cerebral ischemia, an experimental study

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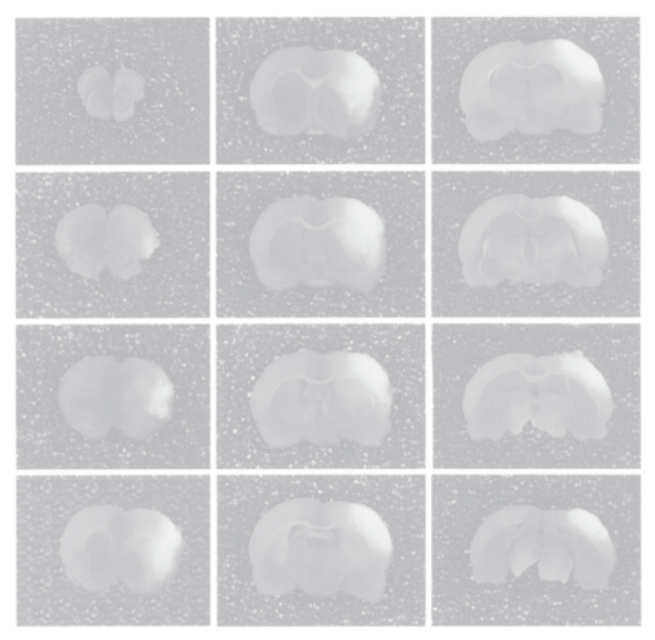
Chapter 1

Introduction



INTRODUCTION

Cerebral ischemia is an important cause of brain damage and can lead to temporary or permanent loss of function in various disease entities. Deficits in essential substrate delivery and insufficient clearance of toxic metabolic waste products both create a harmful environment for neural structures and their surroundings. In-depth knowledge of the pathophysiological mechanisms and cascades activated during this process will help to develop therapeutic strategies. An array of different experimental in vivo and in vitro models is available to study the pathophysiology of cerebral ischemia, all mimicking various types of ischemia (global versus focal). Although in vitro models provide more controlled environments, they cannot incorporate important outcome determining factors such as auto regulation and microvasculary changes. For this purpose, in vivo models are far more suitable. Considerations that should be made before using existing in vivo models include the type of ischemia studied and the reproducibility of the ischemia. This reproducibility, which has been found to be affected by many factors like animal strain[18], vendor[18], location and duration of arterial occlusion and anesthesia technique[25], is the first topic of this thesis.

NO production is a cardinal step in the pathophysiology of cerebral ischemia[5,7]. The discovery of NO as an endothelium-derived relaxing factor (EDRF) revolutionized thinking about cell to cell communication. Its importance was confirmed with a Nobel price for Robert Furchgott, Louis Ignarro and Ferid Murad in 1998. NO production was found to be an important mediator of ischemic damage[21]. On the other hand NO's powerful vasodilator action and inhibition of platelet aggregation contributes to collateral flow, thereby reducing ischemic damage. Different sources of NO production have been identified in the different isoforms of the enzyme: neuronal NO-Synthase (nNOS), endothelial NO-Synthase (eNOS) and inducible NO-Synthase (iNOS). In cell cultures, NO was found to be toxic in a dose dependent way[8], but in vivo the effect varies from detrimental to neuroprotective[12]. Since direct measurements of NO, which in an oxygen-containing environment has a very short half-life in the order of seconds[13], are complicated, NOS inhibitors and NO donors have been used to study the role of NO in the pathophysiology of cerebral ischemia. Information on the mechanisms and actions of NO has been largely derived from studies in which NOS inhibitors have been used in vivo, but published reports have shown contradictory results, with both cerebroprotective and detrimental effects of NOS inhibition[3]. A comparison of the effects of NO modulators in vivo between different investigations is complicated by differences in methodology, including animal model, anesthetic agent, occlusion technique, duration of ischemia, and drug dosing. To reduce the risk of alternative, a-specific effects of the inhibitors and donors, we set out to

compare the effects of two different NOS inhibitors and NO donors in a focal temporary ischemia model.

Polyamines with NO donor capacity reduce ischemic injury[20]; while in general they are important for cell growth, differentiation[23] and numerous cellular functions including protein phosphorylation and protein synthesis[16], regulation of gene expression, programmed cell death, inhibition of the mitochondrial inner membrane permeability[15], regulation of mitochondria Ca^{2+} transport[17], free radical scavenging[24] and the regulation of nitric oxide synthase (NOS)[11]. The role of the polyamine part itself in the pathophysiology of cerebral ischemia has not been elucidated. Following either transient global, focal, or focal permanent cerebral ischemia, there is a significant increase in polyamine metabolism with increased ornithine decarboxylase (ODC) activity and increased putrescine levels[19], which is subsequently metabolized to spermidine and spermine[22]. It has been demonstrated that spermine concentrations in brain are either slightly reduced[14] or moderately increased[14] following global cerebral ischemia. In focal cerebral ischemia, significant reductions or no alterations in spermine concentrations have been demonstrated[4,19]. Since the polyamine may contribute to the protection against ischemic damage, we tested the effects of exogenous spermine on stroke size in a focal cerebral ischemia model.

Although variable results of NO modulations in experimental focal cerebral ischemia[1] can be partly explained by previously discussed variables like strain, dosing and model, we investigated the effect of intra-ischemic intracellular pH (pHi). The activities of endothelial and neuronal NOS enzymes have been shown to be dependent on brain intracellular pH (pHi), with an optimal enzyme activity at a pH of 7.6 for (microsomal) eNOS[10] and at a pH of 6.7 for nNOS[9]. The hypothesis is tested that the severity of ischemic brain acidosis affects the activity of the NOS enzyme and with this its contribution to ischemic damage. As it is not possible to measure brain pH and NO simultaneously in vivo, given the current technology, the neuroprotective effects of NOS inhibitors and NO donors were used to study the effect of pHi on the production of NO. The degree of brain acidosis was manipulated by altering serum glucose concentrations[2,6].

In this thesis the following questions are addressed:

- **Does the severity of ischemia affect the reproducibility of cortical infarction in a temporary focal cerebral ischemia model in the Wistar rat (chapter 2)?**

- **How do the effects of NOS inhibition compare to NO donor treatment in this focal cerebral ischemia model (chapter 3)?**

- **Can spermine contribute to the protective effects of the NO donor spermine NONOate (chapter 4)?**

- **Can intracellular pH explain the reduced effectivity of NOS inhibition in more severe cerebral ischemia (chapter 5)?**

- **Do the observed effects of NO donor treatment in more severe cerebral ischemia comply with the effect of pHi on the NOS enzyme (chapter 6)?**

To discuss subsequent developments chapter 7 addresses the transfer of knowledge from laboratory to clinic and the possibles roles for NO in future therapies. Chapter 8 contains the summary.

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