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Hemoglobin in the rodent brain

Geboren am 20.10.1978 in Baden-Baden
Examen am 11.01.2005 (Diplombiologie) an der Universität Heidelberg
Promotionsfach: Physiologie
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Neurons are metabolically expensive high-performance cells that consume peak levels of oxygen and glucose. In the rodent brain, the signaling related energy use of ~ 30 µmol ATP/g/min equals that of human leg muscle running marathon. As a special adaptation to their costly metabolism, oxygen-binding myoglobin is expressed in muscles. Myoglobin improves the recruitment of oxygen from the blood to the muscle cells, thus optimizing the aerobic use of their fuels.

In analogy to myoglobin, oxygen binding globins were also discovered in neurons. In invertebrates, the existence of nerve globin has been described but it has not been genetically defined. The nerve globin of the clam *T. alternata*, for example, has the ability to maintain oxygen supply and neuronal function for 30 minutes in an oxygen-free atmosphere. In the vertebrate brain the only known members of the globin family are neuroglobin (Ngb) and cytoglobin (Cygb). A contribution of these to oxygen transfer in the brain has not been demonstrated so far.

The present study had the aim to answer the following questions:

1. Is hemoglobin (Hb), known as the oxygen carrier of the blood , expressed in neurons ?

2. Is neuronal Hb regulated by the erythroid growth factor erythropoietin (EPO) ?

3. What could be the function of neuronal Hb ?

Ad 1.: In the past, Hb has been found in brain samples. However, these findings have so far been exclusively interpreted as an artifactual contamination by residues of blood Hb. Here, it was demonstrated by immunohistochemical stainings that Hb is expressed in neurons of the cortex, hippocampus, olfactory bulb and cerebellum of rodents, but not in glial cells. The subcellular localization of Hb was compared to that of Ngb. Hb was distributed mainly along the dendrites and axons, whereas Ngb was located in the perinuclear somata of the neurons. The quantification of the Hb concentrations in the neurons based on elaborate immunohistochemistry showed that subcellular concentrations of Hb in the dendrites and axons are in the milli-

molar range. Thus they are comparable to the concentrations determined for the invertebrate nerve globins.

In primary cultures of rat neurons and in neuronal progenitor cells, the Hbα and Hbβ subunits were detectable on the mRNA and protein level, even after more than 20 self-renewing passages in serum-free medium. This ruled out the possibility of red blood cell contamination in the brain sections.

Ad 2.: An up-regulation of the neuronal Hb expression could be demonstrated in cell culture by transgenic overexpression. In vivo, a single dose of EPO was injected i.p. The neuronal Hb expression in the mouse brain was increased within 24 h. At the same time the tissue oxygenation in the brain was enhanced under both normoxic and hypoxic conditions. This finding indicates a connection between the increased levels of neuronal Hb and enhanced tissue oxygenation.

Ad 3.: The function of neuronal Hb was tested in vitro and in vivo. In vitro, no overexpression was achieved using DNA vectors that contained the Hb coding sequences alone. As an alternative to transgenic overexpression native Hb protein was delivered to the neurons using a new method of proteofection. Although Hb could be successfully transferred these experiments but did not provide evidence for a functional role of Hb in cell culture.

In the in vivo studies, a negative relationship was found between cellular oxygen levels and Hb content. Hb-rich neurons were found to be less hypoxic than neurons of low Hb-content. Evidence was provided that Hb-positive cells are oxygen privileged, as they can be sharply distinguished from neighboring cells with a low pO_2 and low Hb-content. This suggests a reciprocal relationship between neuronal Hb levels and tissue oxygenation.

In conclusion, the findings of the present study show that besides neuroglobin and cytoglobin, Hb is also expressed in the neurons of mice and rat. The sub-cellular expression levels of neuronal Hb in dendrites and axons are in the range of the neuronal globin-concentrations that have been described in invertebrates in which they appear to be involved in oxygen storage. The reciprocal relationship between brain Hb and degree of tissue hypoxia which is shown in the present study suggests that a similar oxygen-related function is mediated by Hb in rodents. The potential of neuronal Hb to recruit oxygen would be based on the absence of 2,3-bisphosphoglycerate. Then, the high oxygen affinity of Hb equals that of myoglobin, enabling the oxygen transfer from blood to neurons at low oxygen levels.

On the basis of the present results it is postulated that neuronal Hb serves as short-term oxygen storage ("oxygen capacitator") that bridges the gap between rapidly rising energy de-

mands upon neuronal activation and the delayed response by the vascular system of the brain that channels blood flow to areas of demand. Furthermore, the regulation of Hb expression in the brain by EPO indicates a new and important role of neuronal Hb in the protection against hypoxia.