Energy metabolism and brain damage
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SUMMARY

In a general sense this thesis comprises three subjects: a) the changes in energy metabolism of the brain during cerebral pathology, b) the effect of alterations in energy metabolism on the extent of brain damage, and c) measures to prevent or limit brain damage. In this context the formation of brain damage and possible restorative processes are studied in two models of cerebral pathology: a) a (modified) Levine model of hypoxic and ischemic brain damage in rats, and b) the freezing lesion in cats; the latter is a model of disruption of the blood-brain barrier (BBB) and formation of vasogenic brain edema. Furthermore, some methods to protect the brain against (hypoxic-ischemic) brain damage are evaluated.

Chapter 1 is a general introduction to this thesis. In the first part the technique of positron emission tomography (PET), which is used to evaluate brain metabolism and brain function, is reviewed briefly. Next, the function of ketone bodies (KBs) in cerebral metabolism and their possible role in cerebral protection are described. The subject of the next section is the formation and evolution of vasogenic brain edema after BBB disruption; an experimental model of cold-induced brain damage is presented. Thereafter, many of the processes playing a role in the formation of hypoxic and ischemic brain damage are summarized. This summary is followed by a review of methods to visualize and quantify cerebral damage. In addition, methods to prevent or limit (hypoxic-ischemic) brain damage are discussed. The final part of this chapter comprises a brief outline of the aims and scope of this thesis. Two of the main goals are: a) to explore the potential of positron-emitting KBs for the evaluation of changes in brain metabolism and BBB function, and b) to assess the possible role of KBs in the prevention of brain damage. Chapter 1 is concluded by a short preview of the structure and contents of this thesis.

In chapter 2 the synthesis and purification of \(^{11}\text{C}\)-acetoacetic acid (\(^{11}\text{C}\)-AcAcOH) are described. The objective of the synthesis of this compound was to study the metabolism of KBs by PET, both under normal circumstances and in cases of cerebral damage, and to evaluate the potential of positron-emitting KBs for the visualization of brain
pathology. 1-\textsuperscript{11}C-AcAcOH was synthesized by carboxylation of the acetone carbanion, and purified by HPLC. The purified product was obtained in radiochemical yields up to 58\% in a total preparation time of 30 min. The distribution of 1-\textsuperscript{11}C-AcAcOH in rats and cats was studied by PET. After an initial rapid uptake of radioactivity from the blood into the tissues, a few minutes after injection a steady state was reached in both species. In cats the relative uptake of 1-\textsuperscript{11}C-AcAcOH into the brain was two to three times larger than in rats, making the cat a more appropriate animal to study cerebral metabolism by PET than the rat. PET scans of the brain of cats with a unilateral freezing lesion were also performed. Three weeks after inflicting the lesion, the uptake of 1-\textsuperscript{11}C-AcAcOH into the damaged, ipsilateral hemisphere was much larger than uptake into the intact, contralateral one. At the same time there was no Evans blue staining of brain tissue, indicating integrity of the BBB, whereas substantial Evans blue staining was observed 1 day after inflicting the lesion. Thus, after initial opening the BBB had recovered completely at 3 weeks. Apparently, the relatively increased uptake of 1-\textsuperscript{11}C-AcAcOH into the ipsilateral hemisphere was not caused by unrestricted diffusion due to BBB disruption, but has to be attributed to enhanced metabolism of KBs.

The primary goal of the experiments described in chapter 3 was to test the potential of 1-\textsuperscript{11}C-AcAcOH for the visualization of cerebral pathology more extensively in the model of experimental brain damage we also used in the previous chapter, viz. the cat with a unilateral freezing lesion. An additional and more general goal was to assess the changes in cerebral uptake and utilization of 1-\textsuperscript{11}C-AcAcOH (reflecting energy metabolism and lipid synthesis), \textsuperscript{18}F-2-fluoro-2-deoxy-D-glucose (\textsuperscript{18}FDG; energy metabolism) and L-(1-\textsuperscript{11}C)-tyrosine (1-\textsuperscript{11}C-Tyr; protein synthesis) shortly after the infliction of the freezing lesion and the subsequent disruption of the BBB, as well as during the period of recovery.

The results of the PET studies using 1-\textsuperscript{11}C-AcAcOH showed that 1 day and 1 week after inflicting the freezing lesion the uptake of radioactivity at the site of the lesion was substantially increased; after 2 and 3 weeks the ipsilateral uptake remained elevated, but this en-
The results of the PET studies using \(^{11}C\)-Tyr were also indicative of restorative processes. The difference between the amount of radioactivity at the site of the lesion and the amount in the corresponding contralateral region increased from virtually nil 1 day after inflicting the lesion to 29\% after 2 weeks. A similar trend was observed outside the area of the lesion. We suggest that the increased uptake of \(^{11}C\)-Tyr into the ipsilateral brain hemisphere was caused by enhanced protein synthesis due to BBB repair.

The PET studies using \(^{18}FDG\) showed that initially the uptake of radioactivity into the area of the lesion was significantly lower than in the corresponding contralateral area. After 2 weeks, however, the uptake of \(^{18}FDG\) into the lesion was no longer different from control values, once again indicating restoration of BBB function. Outside the area of the lesion initially the amount of radioactivity was similar to the radioactivity found in the corresponding undamaged, contra-
lateral area. After the first week, however, an increasing difference in uptake of $^{18}$FDG between the ipsi- and contralateral areas became apparent, reaching a maximum at 3 weeks (-14% ipsilaterally). The phenomenon of reduced metabolic activity thus had spread from the site of the lesion into other parts of the ipsilateral hemisphere. This reduction may be attributed to the loss of neuronal input from the damaged cells in the lesion into the cells of the deeper-lying brain areas.

In conclusion, the following factors govern the cerebral uptake (and utilization) of $^{11}$C-AcAcOH, $^{11}$C-Tyr and $^{18}$FDG: a) BBB opening and the concurrent increase of unrestricted influx of substrates into the brain, and decrease of carrier-mediated diffusion, b) loss of brain cells (first in the area of the lesion and later on in the deeper-lying areas), and c) restoration of BBB function. The relevance of each of these factors is different for each of the three radiopharmaceuticals used. Moreover, their relevance depends on the time passed since the infliction of the lesion.

In chapter 4 the effect of the sodium channel blocker tetrodotoxin (TTX) is evaluated in two models of brain pathology. TTX is an example of a potentially protective substance intervening at the neuronal level. The first model we used was an acute model comprising cardiac arrest in rats. The second model was a survival model, viz. a modification of the Levine model. The main parameter of brain damage was the change in the concentrations of cations in brain tissue. In the acute model the interstitial brain potential was recorded. The deflection of the brain potential, which is caused by changes in cerebral cation concentrations, was substantially delayed after local administration of TTX. In the survival model the combined effect of hypoxia and ischemia produced unilateral cerebral infarcts, characterized by a decrease of the concentrations of K$^+$ in the brain and an increase of Na$^+$ and Ca$^{2+}$ concentrations. This result was confirmed by other parameters such as survival rate, Evans blue staining and edema formation. The striatal cationic changes in the modified Levine model were largely prevented by local injections of TTX. These results led us to the following conclusions: a) TTX can, at least in part, prevent the deleterious processes induced by hypoxia and ischemia; b) the
evaluation of protective methods in the acute model may provide an indication of the results to be expected in the more laborious survival model; and c) the results in the acute model suggest that the protective effect of TTX may be attributed to a delay in cell depolarization, so that the duration of the polarized state is shortened. The final conclusion is that the influx of Na⁺ into brain cells, and thus neurotransmission, may play a crucial role in the development of cerebral damage.

The principal aim of the experiments described in chapter 5 was to test the protective potency of both starvation and hypothermia (two methods acting primarily at the metabolic level) in the prevention of brain damage. Hence, fed and starved normothermic and fed hypothermic rats were subjected to an experimental procedure derived from the Levine model of hypoxic-ischemic brain damage. During the experiment the physiological parameters of blood and the mean arterial blood pressure (MABP) were measured repeatedly. Cerebral damage was assessed on the basis of the changes in regional cation concentrations of the brain. The experimental procedure produced unilateral brain infarcts, characterized by increases of the Na⁺ and Ca²⁺ concentrations and concomitant decreases of the K⁺ and Mg²⁺ concentrations, thus substantially increasing the Na⁺/K⁺ ratio. These changes were, at least in part, prevented by starvation and hypothermia. The results of other methods to assess brain damage (the determination of survival rate, of Evans blue scanning, and of brain water content) confirmed the protective action of starvation and hypothermia. During hypoxia the arterial oxygen pressure (pO₂) in starved and hypothermic rats was not significantly higher than in control rats. This result indicates that starvation and hypothermia do not attenuate the severity of the initial hypoxic insult. We suggest that the basic mechanism behind the protective effect of both starvation and hypothermia is preservation of energy substrates, either by depressing the rate of energy-demanding processes (as occurs during hypothermia) or by providing an alternative energy substrate (KBS) in addition to glucose (as happens during starvation). Thus, the deleterious processes induced by hypoxia and ischemia (e.g., depletion of energy substrates, formation of lactate, etc.) will be prevented. Finally, both starvation and hypo-
thermia largely prevented the characteristic reduction of the MABP during hypoxia, thus maintaining an adequate perfusion of the brain. This result indicates that maintenance of cardiac function contributes to the protective effect of starvation.

In chapter 6 the mechanism of starvation-induced protection of the brain against hypoxic-ischemic damage is investigated more extensively. Groups of fed and starved rats were infused with a solution of either saline, KBs (β-hydroxybutyric acid; B-OHB) or glucose and subjected to unilateral carotid artery occlusion and experimental hypoxia, according to a procedure derived from the Levine model. In all experimental groups pO₂ was equally reduced during hypoxia; changes in the other parameters (pCO₂, pH, etc.) showed only minor discrepancies between groups, if any at all. However, Evans blue staining of brain tissue occurred more frequently in saline- and B-OHB-infused, fed animals than in the corresponding starved animals. Notably, the extent of Evans blue staining in fed and starved, glucose-infused animals was not significantly different. There was also no difference between the three groups of fed animals. These results indicate that starvation provides protection; infusion of glucose appears to abolish this protective effect. In fed animals the effect of infusion of B-OHB on Evans blue staining was nil, suggesting that elevation of KB levels does not offer much protection. These conclusions were supported by the results of cation analysis. Generally, cerebral damage, as quantified by the changes in cerebral cation concentrations, in the groups of starved rats was less than in the groups of fed rats. Glucose infusion appeared to abolish the cerebral protection in starved rats. Cation analysis also revealed that in fed animals cerebral damage is aggravated by infusion of glucose. The effect of B-OHB infusion appeared to be nil, both in fed and in starved rats. The conclusions from these results are: a) starvation protects the brain against damage caused by the combined effect of ischemia and hypoxia; b) elevation of blood glucose levels aggravates the hypoxic-ischemic damage and may completely eliminate the protection offered by starvation; and c) raising blood KB levels by infusion of B-OHB does not contribute substantially to cerebral protection.

The concentrations of B-OHB, acetoacetic acid (AcAcOH) and glucose in
the MABP brain contributes function of solution glucose and experimental model. In hypoxia; only minor trans blue and animals. Cred, glucose also results if glucose the effecting that analysis. cerebral less than in Polish the revealed of glu- in fed and starvation effect of aggravates the protective infusion action. glucose in

blood, and the B-OHB content of the brain were also determined. As expected, starvation increased blood KB levels, whereas blood glucose levels were reduced. KB concentrations of the brain appeared to be enhanced too. Infusion of B-OHB led to elevated KB levels both in blood and in brain tissue, whereas blood glucose levels were unaffected. Finally, infusion of glucose appeared to reduce the concentration of KBs in the blood. Since B-OHB infusion does not reduce cerebral damage, it may be concluded that the increased availability of KBs does not result in a (metabolic) shift from glucose to KBs as the main energy source of the brain; thus lactate production during hypoxia will be the same as before infusion of B-OHB. The finding that blood glucose levels are unaffected by B-OHB infusion leads to the same conclusion. Therefore, raising the concentrations of KBs in the body will be of little therapeutic relevance, if the glucose concentrations are not reduced at the same time. This very state of hyperketonemia combined with hypoglycemia is accomplished during starvation. At the end of the chapter some other factors that may be relevant to the protective action of starvation (peripheral effects; changes in the release of hormone-like substances, such as insulin) are discussed.