Hibernation, Starvation and Hypothermia – Models for Alzheimer's Disease

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Alzheimer's disease belongs to a class of neurodegenerative disorders called "tauopathies" with neurofibrially degeneration associated with the formation of tangles which major component are paired helical filaments (PHF) of hyperphosphorylated tau protein. Tau protein is a microtubule associated protein and its abnormal high degree of phosphorylation in diseased brains is associated with reduced affinity for microtubules, translocation from the neuritic to the somatodendritic compartment. Under normal conditions it is involved in microtubule stabilisation and thereby in the mainteance of axonal transport between soma and presynapse. Loss of microtubule stabelising function as a result of phosphorylation and aggregation is thought to be followed by synaptic regression and cognitive decline long before tangle formation and cell death. Research mainly focused on genetic factors involved in the pathogenesis of tauopathies has lead to the dicovery of several mutation in "familiar" forms, e.g. in the genes coding for amyloid precursor protein, presenilin or tau, which alltogether account for less then 1% of all cases. In contrast to the patients, carrying the mutation, the transgenic mice harboring these mutations do not express the full phenotype of Alzheimer's disease (Gärtner and Arendt, 2006). Additionally, these models cannot explain the etiology in the more frequent "sporadic" forms. Therefore we intend to follow an alternative approach searching for physiological models of tau hyperphosphorlyation.

Models of tau hyperphosphorlyation under physiological conditions

Previously we observed a PHF-like hyperphosphorylation of tau protein in hibernating ground squirrels (Arendt et al., 2004) and could confirm these results in hibernating Golden hamsters

(Härtig et al., 2007). By immunohistochemistry a similar distribution of PHF-like phosphorylated tau can be demonstrated in cell types and regions highly vulnerable to neurodegeneration in Alzheimer's disease, e.g. the entorhinal cortex, hippocampus (Arendt et al., 2004) and the cholinergic neurons of the basal forebrain (Härtig et al., 2007). Similarly to hibernation, PHF-like hyperphosphorlyation of tau was observed in starving mice (Yanagisawa et al., 1999). Both physiological *in vivo* models are states of hypometabolism associated with a marked reduction in the overall metabolic rate and profund hypothermia with the body temperature shifting towards ambient temperature. Western blotting shows that the phosphorylation of tau protein includes several Alzheimer's disease relevant epitopes, is significant in both models and, most importantly, reversible after rewarming (**figure 1**).

Mechanisms of tau hyperphosphorylation

Serin/Threonin phosphorylation of tau is regulated by several kinases, e.g. cyclin dependent kinase 5 (cdk5), glycogen synthase kinase 3 beta (GSK3b), mitogen activated protein kinase (MAPK) and stress acitivated kinases, as well as by phosphatases, mainly protein phosphatase 2A (PP2A). A series of publications (Yanagisawa al., 1999; Planel et al., 2001; Planel et al., 2004) clearly demonstrated that starvation in mice at room temperature causes hypothermia. During hypothermia enzyme activity of both phosphatases and kinases is diminished, but changes are less pronounced for kinases. Therefore, kinase activity overrides phosphatase activity leading to a hyperphosphorylation of tau. Similarly, in cell cultures transfected with wildtype tau this hyperphosphorylation can be induced by hypothermia while hypometabolism alone is not sufficient (**figure 2**). In hibernation tau hyperphosphorylation might not be solely caused by hypothermia but also by an adaptation of enzyme kinetics, e.g. an increase in kinase activity at lower temperatures in brain extracts from hibernating animals compared to those from non-hibernating animals.

Is tau phosphorylation responsible for memory deficits or is it an epiphenomenom?

Hibernation appears to differentially erase memory traces (McNamara and Riedesel, 1973; Zhao et al., 2004; Millesi et al., 2001) but also to improve learning after hibernation (Mihailovic et al., 1968; Weltzin et al., 2006). Unfortuneatly these conflicting results have been obtained in different species and use diverse behavioural tasks which are not comparable directly. It could be argued that these diverse effects are associated with a specific phosphorylation pattern of tau during torpor and arousal states of hibernation. Whether tau hyperphosphorylation caused by starvation induced hypothermia is associated with such deleterious effects on learning and memory is unknown, because all the studies on starving mice (Yanagisawa al., 1999; Planel et al., 2001; Planel et al., 2004) or recently in mice with anesthesia-associated hypothermia (Planel et al., 2007) are lacking any behavioural tests.

Is synaptic plasticity responsible for memory deficits?

Synaptic contacts within the hippocampus are reduced during torpor and been reestablished during arousal (Popov and Bocharova, 1992; Arendt et al., 2004; von der Ohe et al., 2007). In non-hibernating animals similar changes were shown *in vitro* using brain slices (Kirow et al., 2004; Roelandse and Matus, 2004) and *in vivo* during anesthesia associated hypothermia (Popov et al., 2007). Somehow contradictory to the synaptic regression, *in vitro* synaptic plasticity present in long term potentiation in hippocampal brain slices *in vitro* is not affected by transient cooling (hamster: Krelstein et al., 1990; rat: Bittar and Muller, 1993). The drawback of these studies might be that these hypothermia phases lasted less than 1 hour which is considerably shorter than the several days spent in torpor. Additionally these *in vitro* preparations include cooling of the tissue to prevent excitotoxic damaged by the glutamate released during slicing, and this cooling already affects synaptic plasticity. Therefore, further experiments on synaptic regression in hibernation should be done by *in vivo* recordings of long term potentation.

What are these model useful for?

These three models mimicking the tau hyperphosphorlyation as seen in Alzheimer's disease and other tauopathies are exceptionally useful in testing therapeutic strategies. Most important, they show a reliable, sustainable, and marked increase in tau phosphorylation at disease relevant epitopes, within hours, days or months, depending on whether hypothermia in cell culture, starving mice or hibernating hamsters are choosen. Pharmacological studies can be carried out without interference from other agents. The cell culture model includes testing of tau translocation from neurite to soma, monitoring of neuronal transport by time lapse microscopy and high throughput screening of therapeutic agents, like tau kinase inhibitors. Even analysis of synapse function could be included into the *in vitro* model by using hippocampal slice culture or dispersed primary cell cultures. Both mice and hamster are easy to maintain and have an intact blood brain barrier as a prerequisite for testing of therapeutic agents. Both models could also be used for behavioural studies in learning and memory, with the advantage that acquisition and recall could be performed without acute effects of hyperphosphorylation, e.g. before and after starvation induced hypothermia or torpor phase in hibernation.

Beyond hypothermia?

Although PHF-like phosphorylation of tau protein was found in both models, a formation of paired helical filaments (PHFs) was not observed. This might be a disadvantage for a model of Alzheimer's disease, but also means that hyperphosphorylation of tau *per se* is not a pathological 'point of no return' and might confer to an physiological 'neuroprotection' against effects of failing energy supply (Arendt et al., 2004). Further studies on the memory deficits after hypothermia are needed – with an emphasis on the role of tau protein and synaptic plasticity. Memory deficits might be the cost on which neuropotection during

hypothermia or hibernation is achieved. Similar, a recent study suggests that post-anesthestic amnesy might be caused by tau hyperphosphorylation through hypothermia (Planel et al., 2007) and might render patients more susceptible to future neurodegeneration.Therefore, we should also be aware of the risks caused by an induced hypometabolic state in humans proposed for emergency, surgery or longtime spaceflights (Biggiogera et al., 2004).

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FIGURE LEGENDS

Figure 1. Reversible phosphorylation of tau in hibernating Syrian hamsters and starving mice.

Western blots were reacted for phosphorylation independent detection of tau (BR134), specific PHF-like tau phosphorylation epitopes (AT8, AT100, AT180, PHF-1, AT270).
EU – eutherm, TE – torpor early, TL – torpor late, AE – arousal early, AL – arousal late, NS – non starved (ad libitum) at room temperature (20°C), SR – starved 2 days at room temperature (20°C), SW – starved 2 days at room temperature (20°C) and subsequent rewarming for 2.5 hours at 37°C.

Figure 2. Tau phosphorylation by hypothermia but not hypometabolism

N2A cells were stable transfected with wild type mice tau. The left blot shows the PHF-like phosphorylation of tau (AT8) after 1 hour incubation at different temperatures. The right blot shows no difference in phosphorylation after 1 hour incubation with 3g/l deoxyglucose (DG) in medium or medium alone (Co) at 37°C.