In Vitro and *In Vivo* Hypothermia Models

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BACKGROUND

Increased and dysregulated phosphorylation of the protein Tau is a well-known hallmark of Alzheimer's disease. Beside other triggers, more and more hints point towards anesthesia-induced hypothermia as novel cause for Tau hyperphosphorylation. Thus, the design and screening of novel drugs as kinase inhibitors are current central AD research strategies. Inducible *in vivo* and *in vitro* models are thus crucial and useful tools for studying effects of such promising central nervous system drugs.

MATERIALS AND METHODS

SH-SY5Y cells (SH) or SH cells overexpressing human mutated Tau were treated with kinase inhibitors and were kept under normo- or hypothermic conditions to induce Tau hyperphosphorylation. Afterwards, total Tau and its phosphorylated species were analyzed in cellular lysates.

C57BL/6J mice received either pentobarbital or vehicle under normo- or hypothermic conditions. After body temperature evaluation, brains were collected and total Tau, its phosphorylated species, kinases and amyloid beta levels were examined.

RESULTS

Total Tau and pThr231 Tau remained unchanged whereas pSer396 and pSer262 Tau levels were significantly enhanced in SH and more pronounced in SH-Tau cells under hypothermic conditions. LiCl, a well-known kinase inhibitor, attenuated the hypothermia-induced hyperphosphorylation for screenings of novel kinase inhibitors.

Pentobarbital-induced hypothermia in wildtype mice leads to hyperphosphorylation of different Tau sites, most likely due to enhanced GSK3beta activity. While total Tau levels remain unaltered under hypothermic conditions, amyloid beta levels in wildtype mice are significantly enhanced.

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RESULTS

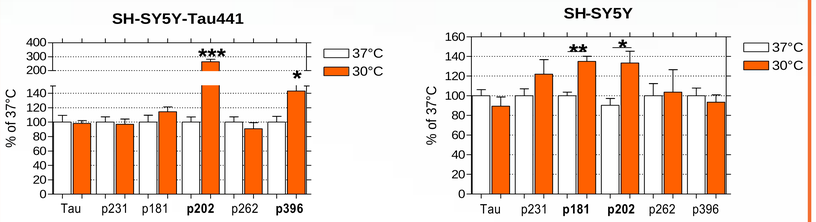

Figure 1. Effects of hypothermia on Tau phosphorylation in SH-SY5Y-Tau441 and SHSY5Y cells. SH-SY5Y-Tau441 or SH-SY5Y cells were subjected to 2h of hypothermia (30° C) or kept at 37° C (normotherm). Effects on Tau phosphorylation sites were determined by immunosorbent assay, respectively. Data are shown as % of 37° C. Statistical significance is indicated by *<0.05, **<0.01, ***<0.001 as determined by t-test (two-tailed, unpaired). Data are shown as group mean + SEM (n=4).

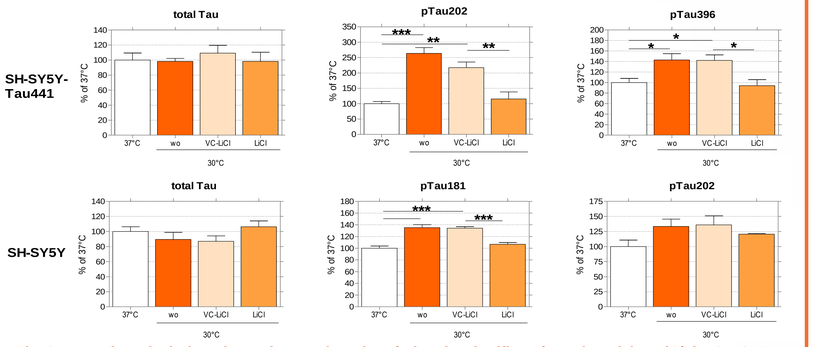

Fig 2. Hypothermia induced Tau hyper-phosphorylation is significantly reduced by LiCl in SH-SY5Y-Tau441 cells. SH-SY5Y-Tau441 cells were subjected to 2h of hypothermia (30° C) and treated with LiCl. Immunosorbent (MSD) quantification is shown of total Tau, pTau202 and pTau396. Data are normalized to normothermic conditions. Data are shown as mean + SEM (n=4). Statistical significance is indicated by *<0.05, **<0.01, ***<0.001 as determined by One-Way ANOVA (Newman's Keuls Multiple Comparison Test).

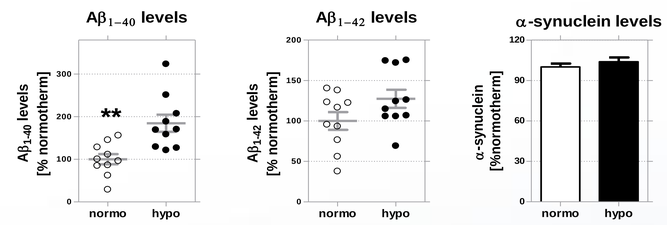

Figure 4. Regulation of Aβ1-40 and 1-42 levels in hemibrains of C57Bl/6 mice under normo- or hypothermic conditions. In total, 20 male animals were injected with pentobarbital and were kept under normo- or hypothermic conditions for 30 min before sacrification and analyses of Aβ species in cerebral homogenates (MSD). Student's t-Tests revealed significant differences between normo- and hypothermic conditions: **p<0.01.

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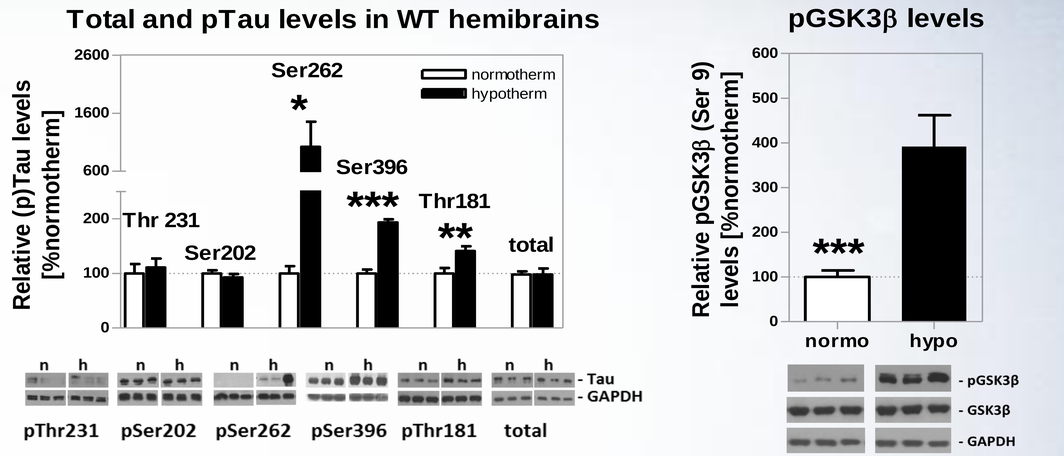

Figure 3. Pentobarbital-induced hypothermia triggers Tau hyperphosphorylation in wildtype mice and is mediated by GSK3β activity. 10 male C57Bl/6J mice of 2 months of age were intraperitoneally injected with pentobarbital and were kept hypothermic (black bars) or normothermic (controls, white bars). Hemibrains were analyzed for Thr231, Ser396, Ser202, Thr181, Ser262 Tau, total Tau, and (p)GSK3β. Densidometric analyses of bands specific for phospho Tau, total Tau, (p)GSK3β and loading controls were performed. Then Tau/phospho Tau and (p)GSK3β expression was normalized to the expression of the loading control. Finally, the levels were expressed as percent normotherm. Statistical analyses (Student's T-test) revealed significant differences between the normo – and hypothermic groups. *p<0.05, **p<0.01, ***: p-value <0.001.

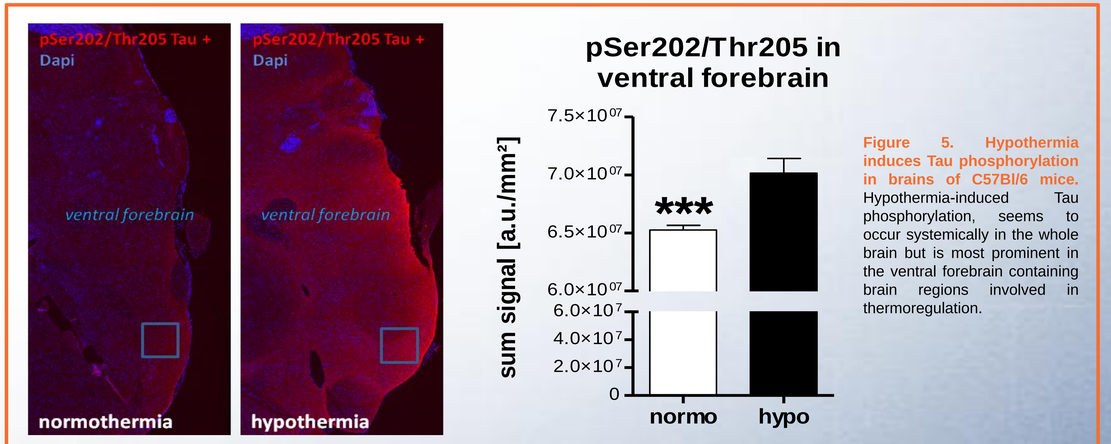

Figure 5. Hypothermia induces Tau phosphorylation in brains of C57Bl/6 mice. Hypothermia-induced Tau phosphorylation, seems to occur systemically in the whole brain but is most prominent in the ventral forebrain containing brain regions involved in thermoregulation.

CONCLUSION

Pentobarbital-induced hypothermia induces Tau hyperphosphorylation and increases amyloid beta production in wildtype mice and leads to Tau hyperphosphorylation in SH and SH-Tau cells. Thus, these non-genetic, acute models are useful, fast and cost-effective tools to study novel drug mechanisms.