HYPOTHERMIA-INDUCED HYPERPHOSPHORYLATION A CELLULAR MODEL TO STUDY TAU KINASE INHIBITORS

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BACKGROUND

Tau hyper-phosphorylation is a known hallmark of Alzheimer's disease (AD). Hence, developing and screening novel kinase inhibitors are in a focus of current AD research. To find promising drug candidates, inducible cellular models of Tau hyper-phosphorylation are useful tools for studying central nervous system drug effects.

MATERIALS AND METHODS

Tau hyper-phosphorylation was induced by hypothermic conditions (2h, 30° C) in SH-SY5Y cells (SH) or SH cells overexpressing the longest isoform of human Tau 441 carrying two well-characterized mutations V337M/R406W (SH-SY5Y-Tau441). To reverse Tau hyperphosphorylation cells were treated with different kinase inhibitors (LiCl, SP600125, Dinaciclib) and were either kept under normo- or hypothermic conditions. Afterwards, total Tau and its phosphorylated species pSer262, pSer202, pSer396 and pThr231 were analyzed in cellular lysates by immunoblot or immunosorbent assay (MesoScale Discovery, MSD).

LiCl and Dinaciclib reduce hypothermia induced Tau hyper-phosphorylation in SH-SY5Y-Tau cells

LiCl, a well-known GSK-3-beta inhibitor, the JNK inhibitor SP600125 and Dinaciclib a Cdk inhibitor were analyzed for their ability to reverse hypothermia induced Tau hyper-phosphorylation in SH-SY5Y-Tau441 cells. LiCl and Dinaciclib significantly reduced Tau hyper-phosphorylation at phospho site 202, already known targets of GSK-3 beta and and Cdk5 and LiCl additionally at site 396. No influences on total Tau levels were detected by immunosorbent assay (MSD assay).

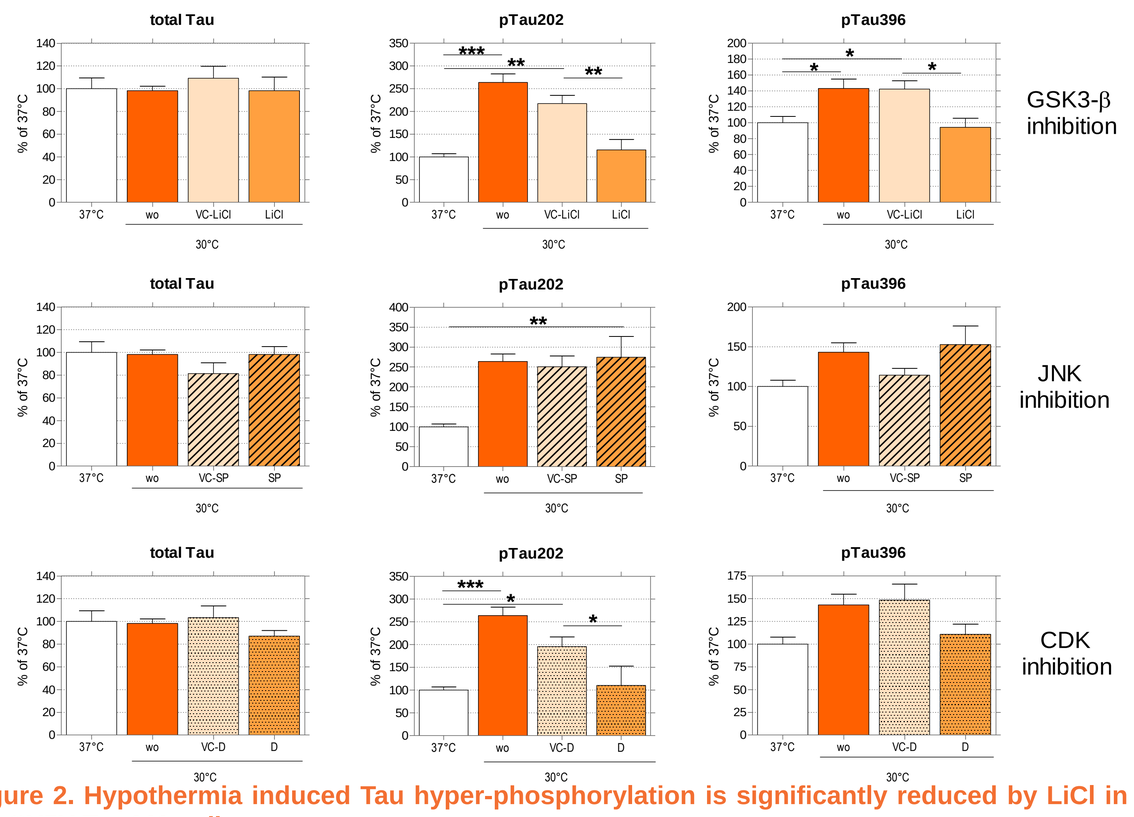

Figure 2. Hypothermia induced Tau hyper-phosphorylation is significantly reduced by LiCl in SH-SY5Y-Tau441 cells. SH-SY5Y-Tau441 cells were subjected to 2h of hypothermia (30° C) and treated with either LiCl (top), SP600125 (middle) or Dinaciclib (bottom) and their corresponding Vehicle Control. Immunosorbent (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).

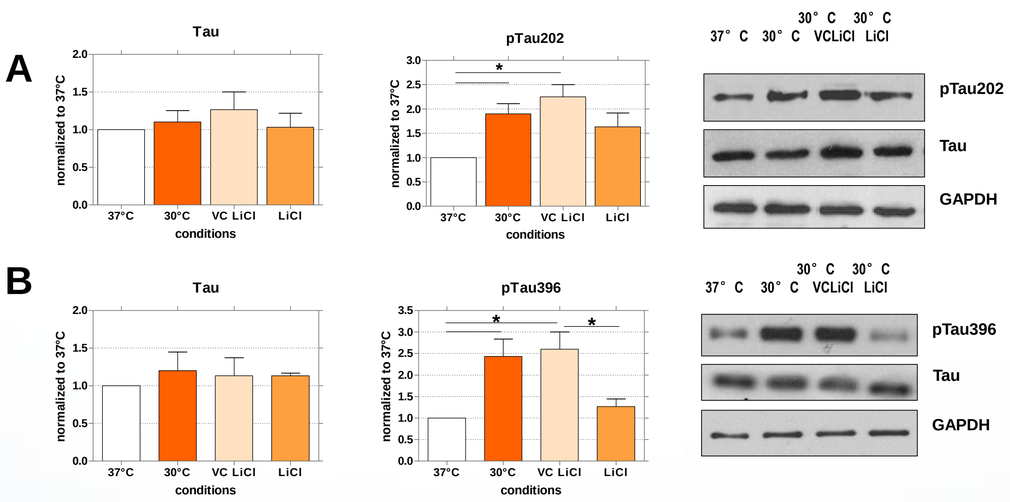

Figure 4: Effects of LiCl on Tau hyper-phosphorylation were confirmed by immunoblot analyses **A)** SH-SY5Y-Tau441 and **B)** SH-SY5Y cells were subjected to hypothermia and treated with either LiCl or Vehicle Control. Immunoblot quantification is shown of total Tau, pTau202 and pTau396. Data are normalized to normotherm conditions. Data are shown as mean +SEM (n=3). Statistical significance is indicated by *<0.05, **<0.01, ***<0.001 as determined by One-Way ANOVA (Newman's Keuls Multiple Comparison Test).

RESULTS

Hypothermia induces Tau hyper-phosphorylation SH-SY5Y-Tau in and SH-SY5Y cells

Tau hyper-phosphorylation was induced by hypothermic conditions (2h, 30° C) in SH-SY5Y cells and SH-SY5Y-Tau441 cells. Total Tau levels and pTau levels at sites Thr231, Thr181, Ser202, Ser262 and Ser396 were analyzed by immunosorbent assay (MSD assay). In SH-SY5Y-Tau441 cells Tau phosphorylation was significantly increased at sites 202 and 396 whereas in SHSY5Y cells significantly elevated levels were detected at site 202 and 181.

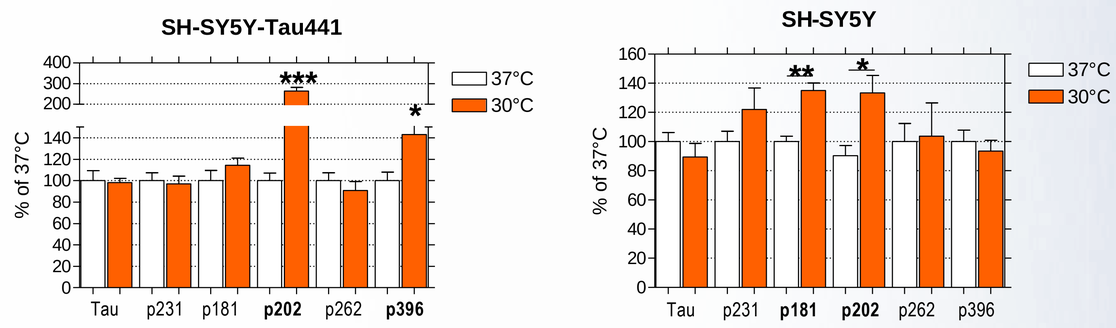

Figure 1. Effects of hypothermia on Tau phosphorylation in SH-SY5Y-Tau441 and SHSY5Y cells. Dinaciclib (0.01 to 15 nM) was applied on DIV8 for 24h to primary rat hippocampal neurons. Effects on Tau phosphorylation sites were determined by immunosorbent assay, respectively. Data are shown as % of 37° 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).

LiCl reduces hypothermia induced Tau hyper-phosphorylation also in native SH-SY5Y cells

LiCl was able to reduce Tau hyper-phosphorylation at sites pTau181 and pTau202 in SH-SY5Y treated cells. No influences on total Tau levels were observed by immunosorbent assay (MSD assay).

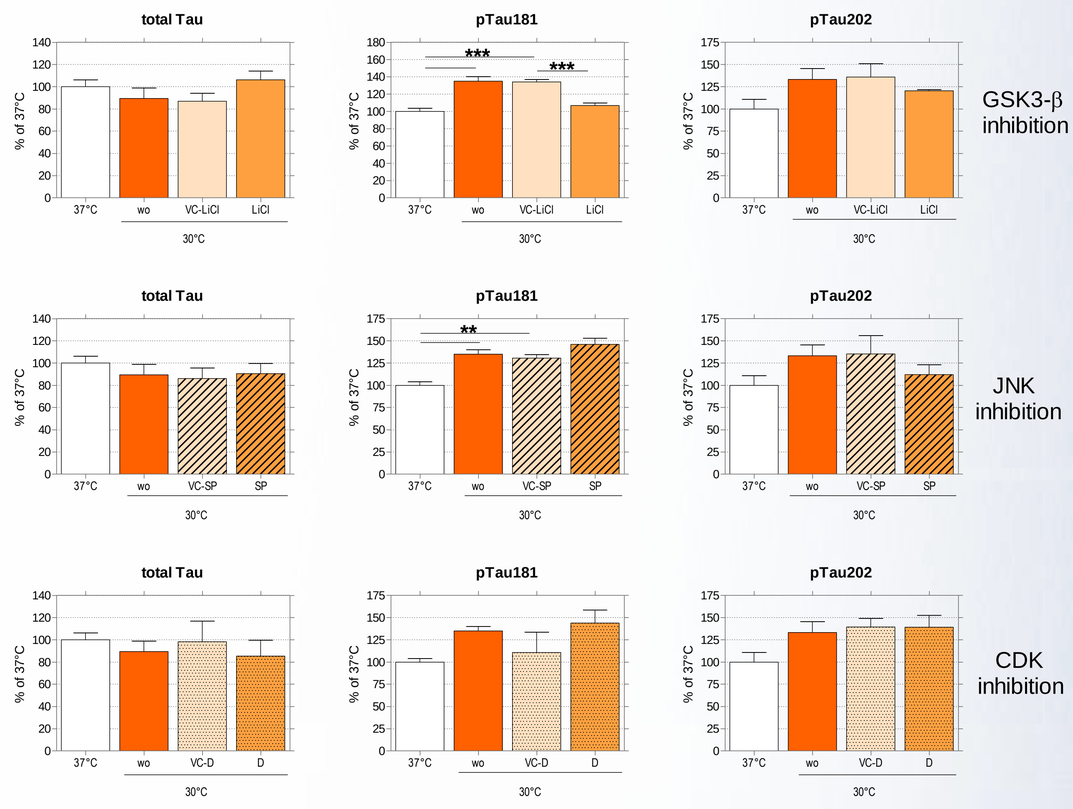

Figure 3. Hypothermia induced Tau hyper-phosphorylation is significantly reduced by LiCl in SH-SY5Y cells. SH-SY5Y cells were subjected to 2h of hypothermia (30° C) and treated with either LiCl (top), SP600125 (middle) or Dinaciclib (bottom) and their corresponding Vehicle Control. Immunosorbent (MSD) quantification is shown of total Tau, pTau181 and pTau202. 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).

CONCLUSIONS

Hypothermia induced a significant Tau hyper-phosphorylation at specific phospho sites in SH-SY5Y and SH-SY5Y-Tau441 cells. This effect could be reversed by kinase inhibitors like LiCl or Dinaciclib. These two *in vitro* models serve as valuable, fast, cost-effective and high-throughput screening tool for studying Tau phosphorylation events or novel kinase inhibitors.

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