Protective effect of hypothermia on ammonia toxicity and energetic disturbances in astrocytes

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Introduction:

Ammonia is a key factor in the pathogenesis of hepatic encephalopathy (HE) due to acute liver failure (ALF). Acute ammonia treatment causes cell swelling and energy failure of astrocytes in HE, which are able to compensate partly by increased anaerobic metabolism and lactate synthesis. On the other hand, astrocytes selectively express glutamine synthetase (GS), which is responsible for ammonia detoxification. It has been the prevailing hypothesis that the osmotic disturbance induced by glutamine leads to astrocyte swelling and consequently brain edema in HE. However, recent studies point to a limited capacity of GS and to direct effects of ammonia on mitochondrial energy metabolism. Mild hypothermia is known to offer protection from severe encephalopathy and lactate accumulation in human ALF. However, the mechanisms are still unknown.

Aims:

Since astrocytes are the primary pathological target in HE, our aim was to investigate if hypothermia protects against ammonia-induced energy failure in cultured astrocytes. Multinuclear NMR spectroscopy was used to evaluate possible underlying mechanisms in this protection.

Methods:

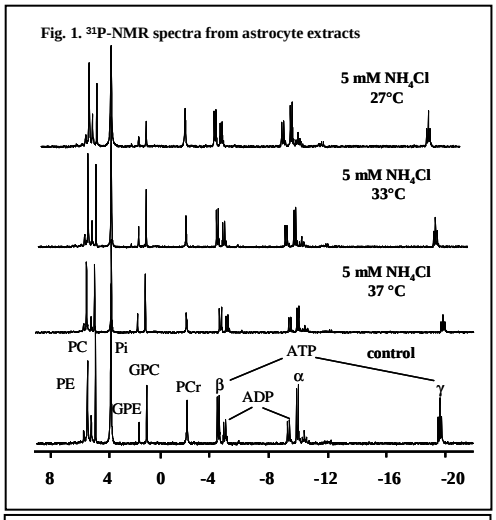
Primary cultures of cortical astrocytes were prepared from 1-2 day old Sprague-Dawley rats and cultivated for four weeks. Then, confluent astrocyte cultures were incubated for 12 hours with DMEM containing 5 mM [1-^{13}C]glucose in the presence or absence of ammonia (5 mM NH_4Cl) under normothermic (37 °C), mild hypothermic (33°C) or moderate hypothermic (27°C) conditions. After removal of the incubation media, the cells were extracted with perchloric acid (PCA) and lyophilized. The lyophilized samples were redissolved in 0.5 ml D_2O and centrifuged. Prior to the NMR analysis, the samples were neutralized with D_2O and NaOH to allow unique chemical shift assignments and to prevent glutamine-carbamate formation in the media. After recording of ^{1}H- and ^{13}C-NMR spectra, samples were treated with EDTA (to form divalent cation complexes) to perform proton-decoupled ^{31}P-NMR measurements. ^{1}H, ^{13}C- and ^{31}P-NMR spectra were recorded on a Bruker WB360 spectrometer.

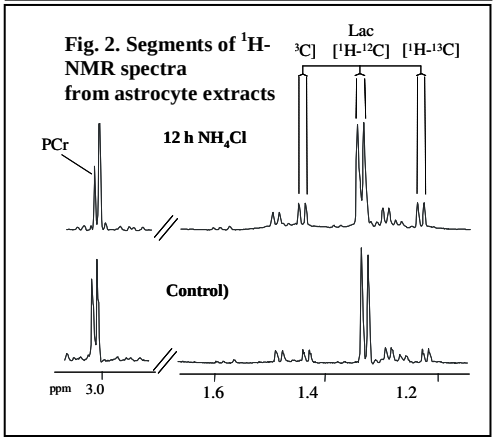
Results:

Cellular energy state. ^{31}P-NMR spectra of PCA extracts depict high-energy phosphates such as nucleoside di- and triphosphates (NDP's, NTP's), in particular ADP and ATP (adenosine di- and triphosphate), and phosphocreatine (PCr). The analysis of these spectra revealed that 12 hour treatment of astrocytes with 5 mM NH_4Cl resulted in decreased ATP- and PCr concentrations to <50% of control values (Fig. 1). The levels of creatine (Cr) (calculated from ^{1}H-NMR spectra, Fig. 2) concomitantly increased to 150% of control, resulting in markedly decreased PCr/Cr ratios to 34% of controls. Mild hypothermia (33°C) alone produced no or only slight changes in high-energy phosphates of astrocytes, but significantly attenuated the ammonia-induced depletion of ATP and PCr under normothermic conditions. While a further decrease in the incubation temperature (from 27°C to 33°C) did not cause a further attenuation of ammonia-induced ATP depletion, PCr levels were completely restored ($p<0.05$).

Glutamine and glutamate. 12 h treatment of astrocytes with NH_4Cl resulted in increased glutamine levels to 159±7.5% of controls. Mild and moderate hypothermia in these cells attenuated the increase in glutamine levels slightly by 6% (ns) and 29% ($p<0.05$), respectively. Its *de novo* synthesis after glucose entry into the TCA cycle by pyruvate carboxylase (PC) (glutamine labelled at C-2) increased to 220±13.4% after treatment with NH_4Cl, leading to an increased percentage ^{13}C enrichment to 139±15% of controls. Mild and moderate hypothermia attenuated this elevation (concentration of ^{13}C-labelled glutamine C2 to 133±13% and 69±2.6% of controls). NH_4Cl treatment increased glutamine synthesis after glucose entry by pyruvate dehydrogenase (PDH) similarly (to 212±33% of controls). Mild and moderate hypothermia led to an attenuation but not to prevention of increased PDH-mediated glutamine synthesis to 166±25 and 139±22% of controls (percentage enrichments in glutamine C4 of 113±25% and 125±24% of controls, resp.). Glutamate synthesis via PC and PDH showed a similar behaviour as glutamine synthesis after treatment with NH_4Cl (increased concentrations of ^{13}C in C2 and C4 to 157±21 and 166±27% of controls, respectively). However, mild and moderate hypothermia led to much lower relative synthesis of glutamate compared to glutamine (to 119±5% and 29±3% of controls for the C2-position and to 103±9% and 71±8% of controls for the C4-position, respectively.), indicating, together with only slight reductions in glutamine levels, a sustanined stimulation of glutamine synthetase.

Glycolysis. As a measure of the glycolytic flux, the *de novo* synthesis of [3-^{13}C]lactate was calculated from ^{1}H-NMR spectra of PCA extracts (Fig. 2). NH_4Cl treatment for 12 h increased the percentage ^{13}C enrichment in intracellular lactate to 130±11%. Mild and moderate hypothermia significantly attenuated this increase to 73±12% and 45±12% of controls, resp.





Conclusions:

The results suggest that hypothermia-induced protection against ammonia toxicity results from a reduced energy demand in cellular reactions uncoupled from mitochondrial glucose metabolism. This leads to a relative inhibition of anaerobic glucose metabolism and a compensatory stimulation of mitochondrial energy production in ammonia-exposed astrocytes.