Synergistic effects of ammonia and manganese on brain cell metabolism

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Synopsis

Similarities between chronic hepatic encephalopathy (HE) and manganese toxicity suggest the involvement of manganese in the pathogenesis of HE. The aim of the present study was to investigate the metabolic response in cultured brain cells (primary astrocytes, neurons and co-cultures) treated with manganese and/or ammonia by means of multinuclear NMR spectroscopy. The data show that ammonia and manganese act in a synergistic way to alter brain cell metabolism. While neurons are particularly sensitive to developing massive energy failure and due to impaired oxidative metabolism of glucose after concomitant incubation with ammonia and manganese, manganese plays a major role in stimulating a protective astrocytic response against mitochondrial energy failure and oxidative stress.

Introduction

Hepatic Encephalopathy (HE) is a disorder than encompasses a spectrum of neuropsychiatric disturbances that can progress to coma. A widely accepted view is that chronic HE is caused by excess ammonia, and therapeutic means are based on the concept of ammonia neurotoxicity [1]. Besides ammonia, manganese accumulates in blood and brain causing pallidal signal hyperintensity in T1-weighted Magnetic Resonance Images [2]. Similarities between chronic HE and "manganism" (manganese neurotoxicity), a brain disorder characterized by extrapyramidal symptoms resembling those of Parkinson`s disease, suggest the involvement of manganese, an important activator and constituent of several TCA cycle enzymes, in the pathogenesis of HE [3]. Hyperammonemia induced metabolic alterations are intensely investigated but experimental data correlate not very well with the abnormal cerebral energy metabolism observed in HE. The aim of this study was to investigate the cerebral metabolic response in cultured brain cells (primary astrocytes, neurons and co-cultures) treated with manganese and/or ammonia by means of multinuclear NMR spectroscopy.

Methods

Cell cultures. Primary astrocytes were prepared from the brains (cortex) of 1-2 day-old rats and cultured for 3-4 weeks in DMEM (10% FBS). Neurons were prepared from 16-18-day-old rat embryos (cortex) and cultured for 5-10 days in BME (0.5 % FBS). For cocultures, astrocytes were cultivated in DMEM (10% FCS). Thereafter, neuronal cells were added and cultivated as described for neuronal cells [4]. The cell cultures were incubated every 24 hours with 100 µM $MnCl_2$ (1 - 5 d) and/or 5 mM NH_4Cl. During the last 12 hours, the cells were incubated with DMEM containing 5 mM [1-^{13}C]glucose in the absence or presence of 100 µM $MnCl_2$ and/or 5 mM NH_4Cl. ***Extraction.*** Cell cultures were washed with ice-cold 0.9% saline and frozen in liquid nitrogen. All samples were extracted and lyophilized as described previously [4]. Di- and trivalent cations were complexed with “Chelex 100”. ***NMR analysis***. The lyophilized samples were redissolved in 0.5-0.6 ml deuterium oxide and centrifuged. ^{1}H-, ^{13}C- and ^{31}P-NMR spectra were recorded on Bruker spectrometers DRX 600 or AVANCE-NB/WB 360. The total metabolite concentrations were calculated from ^{1}H-NMR spectra; the heteronuclear spin-coupling pattern was used to calculate the fractional ^{13}C-enrichments in lactate and glucose. The fractional ^{13}C-enrichments amino acids were calculated from ^{13}C-NMR spectra [4].

Results

^{31}P-NMR spectra of the cell extracts showed that after 3 h treatment of astrocytes with 5 mM NH_4Cl, PCr and NTP concentrations decreased to 29% and 67% of control, respectively. While ATP completely recovered after 5 days of exposure, PCr remained at 74% of control values. 5 d treatment with 100 µM $MnCl_2$ did not result in a decreased energy status of astrocytes, whether ammonia was present or not. In cultured cortical neurons, on the other hand, chronic exposure to ammonia or manganese each caused a significant decrease of PCr and of NTP up to 32% and 64% of control, respectively, which deteriorated further up to non-detectability of PCr concentrations after combined treatment. Concomitant with stimulated uptake and consumption of [1-^{13}C]glucose by either ammonia or manganese, significant increases in the synthesis and release of ^{13}C-labelled lactate (up to 200% increased extracellular lactate) were observed in primary astrocytes. This effect was additive after concomitant treatment with ammonia and manganese (4-fold increase of extracellular [3-^{13}C]lactate). In parallel, oxidative glucose metabolism was stimulated by ammonia or manganese, as indicated 3-fold increased synthesis of [4-^{13}C]glutamate from [1-^{13}C]glucose (through pyruvate dehydrogenase (PDH)), as well as by increased TCA cycle turns and ^{13}C-enrichment in acetyl-CoA entering the TCA cycle (indicated by elevated ratios of double/mono-glutamate C4 and C3, respectively; $p<0.01$) (Fig. 1). These effects of ammonia and manganese on astrocytic mitochondrial energy metabolism were additive resulting in a more than 3-fold stimulation of the TCA cycle turnover compared to their single effects. In contrast to astrocytes, in cortical neurons, uptake and consumption of glucose were decreased by > 30% after treatment with ammonia or manganese. While no changes in lactate synthesis occured, overall mitochondrial TCA cycle metabolism was impaired by > 25%. Concomitant treatment with ammonia and manganese resulted in complete inhibition of synthesis of TCA-cycle related amino acids, such as glutamate and aspartate. These cells also exhibited several distinct morphological features of apoptotic cell death, which was not observed when co-cultured with astrocytes concomitantly with significantly improved mitochondrial energy metabolism. Apart from stimulated mitochondrial metabolism, the more than 10-fold increased *de novo* synthesis of glutathione in astrocytes may offer some protection against energy failure and oxidative stress.

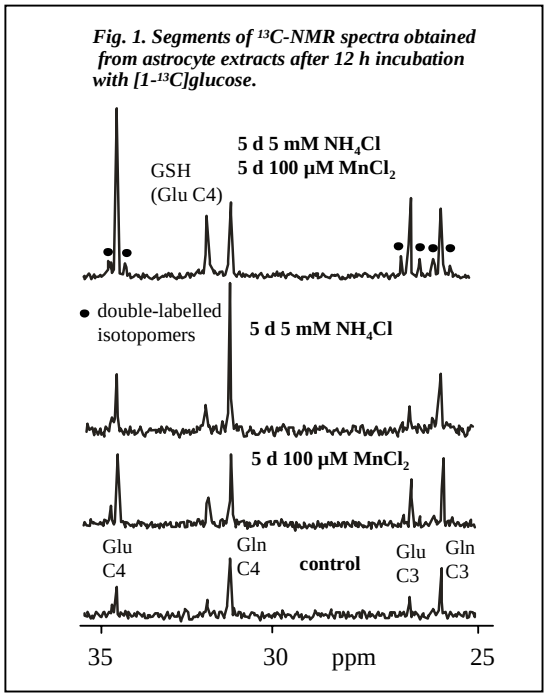

Fig. 1. Segments of ^{13}C-NMR spectra obtained from astrocyte extracts after 12 h incubation with [1-^{13}C]glucose.

Conclusions

These findings suggest that neurons are particularly sensitive to developing energy failure due to impaired oxidative metabolism of glucose following exposure to ammonia and manganese, resulting in disruption of mitochondrial energy production associated with the depletion of high-energy phosphates. In contrast, astrocytes are able to respond by increased oxidative and anerobic metabolism, as well as stimulated glutathione *de novo* synthesis, in order to maintain their normal energy status and to protect against oxidative stress/apoptosis. In addition, in co-cultures, astrocytes may offer some degree of protection against neuronal energy failure and death. The observation, that ammonia and manganese act in a synergistic way to alter cerebral energy metabolism and astrocytic-neuronal communication gain new insights into the consequences of manganese accumulation in the brain in chronic hepatic encephalopathy.

References

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