Extracellular Acidification and Hypoxia in Glial Cell Lines studied by NMR Spectroscopy: Role of Na+/H+ Exchange Subtype 1 Inhibition

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INTRODUCTION

Ischemic tissues suffer from an intra- and extracellular acidification. During ischemia glucose and oxygen deprivation and the lack of detoxification lead to increased carbon dioxide tense and lactate accumulation. Consequently, pH values drop to 6.0-6.3. In general, acidosis depresses metabolic reactions and modulates membrane processes such as dissipative and active transport of ions.[3] Na+/H+ exchange seems to be the predominant mechanism by which glial cells regulate their intracellular pH (pHi) in an acidified medium[4] as shown with the Na+/H+ exchange inhibitor amiloride[5]. In the present study, with respect to selectivity and pharmacology have demonstrated that amiloride unselectively inhibits most plasma membrane Na+/H+ transport systems, i.e., Na+ channel, Na+/Ca2+ exchanger and all four subtypes of the Na+/H+ exchange[6].

METHODS

Approximately 10^3 cells were incubated with Krebs-Henseleit buffer containing 5 mM [1,1’]-glucose at 37 °C. Different extracellular pH values were set by means of bicarbonate buffers and the CO2 content in the air. An extracellular pH (pHe) of 7.4 was adjusted with 26 mM NaHCO3 and 5% CO2/95% air, pHi 6.4 with 6 mM NaHCO3 and 20% CO2/80% air. Incubation with amiloride was kept for two hours. Subsequently, buffers were exchanged and pHe recovery with pHi, 7.4 lasted ten or twenty minutes. Then, cells were extracted. Hypoxia was induced by gassing the Krebs-Henseleit buffer (pHi 7.4) before application and the incubation chamber with 95% N2/5% CO2 in a humidified atmosphere at 37 °C. The incubation buffer (KHB) contained 5 mM [1,1’]-glucose. These conditions were kept for 2 hours, followed by extraction of the cells. After removal of the medium the cells were washed immediately twice with ice-cold isotonic saline, frozen in liquid nitrogen and extracted with 12% perchloric acid (PCA). Neutralized cell extracts were prepared for NMR spectroscopy as previously described[7-9]. NMR spectra were recorded on Bruker AMX 360 and AM 360 NMR spectrometer using 5mm selective probe[20].

RESULTS AND DISCUSSION

An extracellular pHe of 6.4 led to a marked decrease of phosphocreatine in C6 and F98 glial cells (fig.6). This even was more pronounced during the additional incubation with 1 mM HOE642. In this case, the PCr/Cr ratio declined to 20% of control (n=3). Nucleoside triphosphate levels remained constant. The subsequent reconstitution of pHe, 7.4 resulted in a complete recovery of the PCr level up to control values within twenty minutes (fig.6). In contrast, there was no PCR restoration in the presence of 1 mM HOE642. The corresponding 31C NMR spectra of the glial cell lines showed largely decreased cytosolic concentrations of labelled metabolites produced from [1,1’]-glucose after the period of extracellular acidification. Enrichments of Ala, Lac, Gln, Glu, Gro-3-P, Pro and Ser were reduced to less than half of the control values. Comonitantly with the pHe recovery the 31C enrichment was restored in all metabolites within twenty minutes. These findings support a reversible metabolic inhibition and a reversible disorder of the phosphorus energy state due to extracellular acidification. The phosphofructokinase (PFK) as the rate-limiting enzyme of glycolysis is deactivated by slightly decreasing pH values[9]. Consequently, a lack of acetyl-CoA develops and reduces all subsequent biochemical pathways. The lack of PCr/Cr recovery after reconstitution of pHe, 7.4 caused by HOE642 indicates the large presence of the NHE-1 in these gloma cell lines. Its inhibition increases the induced intracellular acidosis and slows down the metabolic recovery from extracellular acidification.

Hypoxia caused a slight increase of both cytosolic concentration and enrichment of Lac (110%, C3: 130% of control, n=3) and a marked increase of Ala (150%, C3: 200% of control, n=3) in F98 glial cells due to higher rates of glycolysis and subsequent regeneration of NAD+ via lactatehydrogenase (LDH)[21]. Gro-3-P levels were twofold elevated during oxygen deprivation because of activation of Gro-3-P-dehydrogenase (Gro-3-P-DH). Additional incubation with HOE642 had no further effect on these metabolite concentrations. The PCr/Cr ratio, however, was decreased after the hypoxic period (50% of control, n=3) (fig.). Levels of cytosolic inorganic phosphate were elevated (140% of control, n=3). Additional incubation with 10 µM HOE642 during oxygen deprivation caused a hardly diminished PCr/Cr ratio (85% of control, n=3) (fig.). No alterations of the nucleoside triphosphate levels were observed. 10 µM HOE642 seem to cause a protection of the energy state during hypoxia because the PCr/Cr ratio almost retained control values (fig.). These data suggest the hypothesis that inhibition of the NHE-1 may have protective effects during cerebral ischemia.

CONCLUSIONS

The present results demonstrate that extracellular acidification causes metabolic inhibition and a reversible depletion of the cellular PCr stores. Subtype 1 specific inhibition of the Na+/H+ exchange aggravates these effects and prevents the cells from reconstitution of control PCr levels obviously due to a prolonged intracellular acidosis. Furthermore, NHE-1 inhibition during hypoxia seems to be beneficial to the energy state of glial cells.

REFERENCES