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 tension 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[1]. Na^+/H^+ exchange seems to be the predominant mechanism by which glial cells regulate their intracellular pH (pH_i) in an acidified medium[2] as shown with the Na^+/H^+ exchange inhibitor amiloride[2]. Recent studies with respect to selectivity and pharmacology have demonstrated that amiloride unspecifically inhibits most plasma membrane Na^+ transport systems, i.e., Na^+ channel, Na^+/Ca^{2+} exchanger and all four subtypes of the Na^+/H^+ exchanger[3,4]. HOE642 (4-isopropyl-3-methylsulphonylbenzoyl)-guanidine methanesulphonate (cariporide) has been characterized as a selective, powerful and tolerable inhibitor of Na^+/H^+ exchange subtype 1 (NHE-1)[5]. The hypothesis that acidosis aggravates ischemic brain damage is controversely discussed. Severe acidosis is linked to enhanced tissue damage[1]. However, milder acidosis or inhibition of the NHE were expected to have protective effects during cerebral ischemia[6]. The NHE-1 inhibitor HOE642 has shown cardioprotective effects in ischemic and reperfused heart models[5]. Therefore, we characterized alterations in glial cell metabolism during extracellular acidosis and subsequent pH recovery in comparison to hypoxia mediated changes. Within these experiments, we studied the role of selective NHE-1 inhibition with HOE642. Data were obtained from ^1H, ^{13}C, and ^{31}P NMR spectra of perchloric acid extracts from F98 and C6 glioma cells.

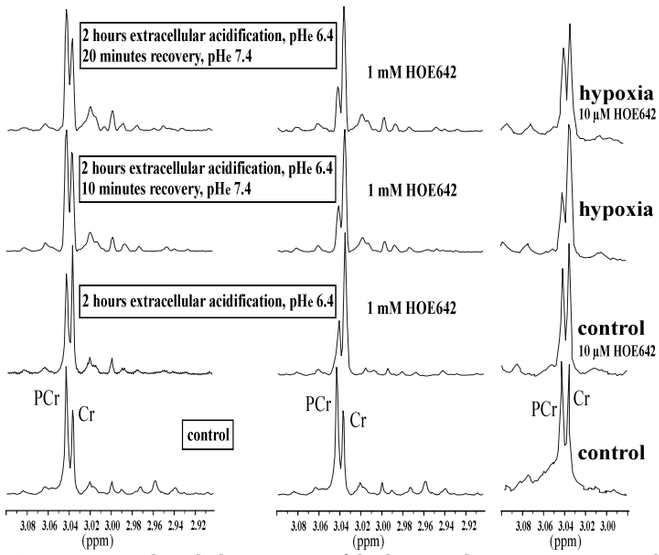
METHODS

Approximately 10^8 cells were incubated with Krebs-Henseleit buffer containing 5 mM [1-^{13}C]glucose at 37 °C. Different extracellular pH values were set by means of bicarbonate buffers and the CO_2 content in the air. An extracellular pH (pH_e) of 7.4 was adjusted with 26 mM $NaHCO_3$ and 5% CO_2/95% air, pH_e 6.4 with 6 mM $NaHCO_3$ and 20% CO_2/80% air. Extracellular acidification was kept for two hours. Subsequently, buffers were exchanged and pH recovery with pH_e 7.4 lasted ten or twenty minutes. Then, cells were extracted. Hypoxia was induced by gassing the Krebs-Henseleit buffer (pH 7.4) before application and the incubation chamber with 95% N_2/5% CO_2 in a humidified athmosphere at 37 °C. The incubation buffer (KHB) contained 5 mM [1-^{13}C]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[4,5]. NMR spectra were recorded on Bruker AMX 360 and AM 360 NMR spectrometer using 5mm selective probes[7,8].

RESULTS AND DISCUSSION

An extracellular pH_e of 6.4 led to a marked decrease of phosphocreatine in C6 and F98 glioma cells (fig.). This was even more pronounced during the additional incubation with 1 mM HOE642. In this case, the PCr/Cr ratio declined to 20% of control (n=3). Nucleosid triphosphate levels remained constant. The subsequent reconstitution of pH_e 7.4 resulted in a complete recovery of the PCr level up to control values within twenty minutes (fig.). In contrast, there was no PCr restoration in the presence of 1 mM HOE642. The corresponding ^{13}C NMR spectra of the glioma cell lines showed largely decreased cytosolic concentrations of labelled metabolites produced from [1-^{13}C]glucose after the period of extracellular acidification. Enrichments of Ala, Lac, Glu, Gln, Gro-3-P, Pro and Ser were reduced to less than half of the control values. Concomitant with the pH recovery the ^{13}C 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 pH_e 7.4 caused by HOE642 indicates the large presence of the NHE-1 in these glioma cell lines. Its inhibition increases the induced intracellular acidosis and slows down the metabolic recovery from extracellular acidification.

Figure: ^{1}H NMR spectra of cell extracts obtained from C6 or F98 glioma cells – extracellular acidification and pH-recovery (left and mid) and hypoxia (right)



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 glioma cells due to higher rates of glycolysis and subsequent regeneration of NAD^+ via lactatedehydrogenase (LDH)[10]. 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 support 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.

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