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Research Report

The effect of acidosis on adenosine release from cultured rat forebrain neurons

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ABSTRACT

During cerebral ischemia, dysregulated glutamate release activates *N*-methyl-D-aspartate (NMDA) receptors which promotes excitotoxicity and intracellular acidosis. Ischemia also induces cellular adenosine (ADO) release, which activates ADO receptors and reduces neuronal injury. The aim of this research was to determine if decreasing intracellular pH (pH_i) enhances ADO release from neurons. Rat forebrain neurons were incubated with NMDA, acetate, propionate, 5-(*N*)-ethyl-*N*-isopropyl amiloride (EIPA) or low pH buffer. pH_i was determined with the fluorescent dye 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM) and cellular release of ADO was assayed. NMDA decreased pH_i and increased ADO release from neurons. Acetate and propionate decreased pH_i and evoked ADO release from neurons. EIPA, an inhibitor of sodium hydrogen exchanger 1 (NHE1), enhanced the acidosis in neurons but did not enhance ADO release. Decreasing extracellular pH (pH_e) to 6.8 or 6.45 significantly decreased pH_i in neurons, but was not consistently associated with increased ADO release. The main finding of this study was that acidosis per se did not enhance ADO release from neurons.

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Abbreviations:
ACE, acetate
AN, adenine nucleotides
ADO, adenosine
BCECF-AM, 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester
CNTs, concentrative nucleoside transporters
CNS, central nervous system
DIV, days in vitro
EIPA, 5-(*N*)-ethyl-*N*-isopropyl amiloride
EHNA, *erythro*-9-hydroxy-nonyl-adenine
ENTs, equilibrative nucleoside transporters
HEPES, 4-2-hydroxyethyl-1-piperazineethanesulfonic acid
INO, inosine
ITU, iodotubercidin
pH_e, extracellular pH
pH_i, intracellular pH
PROP, propionate
MA, methylamine
NMDA, *N*-methyl-D-aspartate
NHE1, sodium hydrogen exchanger 1

1. Introduction

Adenosine (ADO) is a neuromodulator in brain. It activates a family of G-protein-coupled adenosine receptors, termed A_1, A_{2A}, A_{2B} and A_3 receptors. In general, A_1 and A_3 receptors are inhibitory while A_{2A} and A_{2B} are stimulatory. ADO is produced in brain through both intracellular and extracellular pathways (Latini and Pedata, 2001). Intracellular ADO formation is linked to ATP consumption and once formed it is released from cells through bi-directional equilibrative nucleoside transporters (ENTs) of which two subtypes exist in the central nervous system (CNS) (Baldwin et al., 2004). Extracellular ADO formation is secondary to cellular release of adenine nucleotides (AN) and their metabolism by a family of ecto-5′-nucleotidases (Zimmermann, 2000).

During ischemia, brain ADO levels increase up to 100 fold (Parkinson et al., 2000; Rudolphi et al., 1992). Concomitantly, there is a decrease in intracellular pH (pH_i) of 0.5–1.0 units in the affected brain regions (Lipton, 1999; Masino and Dulla, 2005; Nedergaard et al., 1991). Normally, pH_i is tightly controlled during synaptic activation. Sodium hydrogen exchangers (NHE) play an important role in stabilizing pH_i, by exchanging intracellular H^+ for extracellular Na^+. The resulting influx of Na^+ is extruded by the Na^+/K^+ ATPase, at the expense of ATP. During ischemia, pH_i regulation fails due to increases in lactate levels and decreases in ATP levels. Furthermore, cerebral ischemia triggers glutamate release, and both glutamate and NMDA decrease pH_i in neurons by a Ca^{2+}-dependent mechanism (Irwin et al., 1994; Yamamoto et al., 1998). An effect of pH_i on ADO and AMP levels has previously been reported in rat skeletal muscle (Cheng et al., 2000; Mo and Ballard, 2000) and brain (Dulla et al., 2005; Phillis and O'Regan, 2002).

Therefore, the aim of this study was to determine if decreased pH_i, similar to that which occurs in ischemia or following *N*-methyl-D-aspartate (NMDA) receptor activation, can promote ADO release from neurons. Rat cultured forebrain neurons were treated with weak organic acids, buffers of reduced pH or an inhibitor of the Na^+/H^+ exchanger. The effects of these agents to promote ADO release and to decrease pH_i were assessed.

2. Results

2.1. *Effect of NMDA on pH_i and on purine release from primary cortical neurons*

There was a rapid and significant decrease in pH_i following the application of NMDA (Fig. 1A). After 10 min treatment, pH_i decreased from 7.23 ± 0.04 in neurons treated with buffer to 7.10 ± 0.02, 7.12 ± 0.01 or 7.08 ± 0.02 in neurons treated with 30, 100 or 300 μM NMDA, respectively.

NMDA significantly increased total [^{3}H]purine release after 10 (Fig. 1B) or 30 min treatment (Fig. 1C). Tukey's post hoc tests revealed that 100 and 300 μM NMDA increased total [^{3}H]purine release from neurons after 10 min whereas 30, 100 and 300 μM NMDA significantly increased total [^{3}H]purine levels released after a 30-min treatment.

TLC was used to measure released levels of AN, INO and ADO following 10- and 30-min treatments. NMDA had no significant effect on AN release (Table 1). INO release was