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Intracellular acidification increases adenosine transport in human umbilical vein endothelial cells

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ABSTRACT

Introduction: Adenosine is taken up via human equilibrative nucleoside transporters 1 (hENT1) and 2 (hENT2) at a physiological extracellular pH (pHo ~7.4) in human umbilical vein endothelial cells (HUVECs). Acidic pHo increases the uptake of adenosine and 5-hydroxytryptamine (5HT) via hENT4 in this cell type. However, modulation of hENT1 and hENT2 transport activity by the pHi is unknown. We investigated whether hENT1 and hENT2-adenosine transport was regulated by acidic pHi.
Methods: HUVECs loaded with a pH sensitive probe were subjected to 0.1–20 mmol/L NH_4Cl pulse assay to generate 6.9–6.2 pHi. Before pHi started to recover, adenosine transport kinetics (0–500 μmol/L, 37 °C) in the absence or presence 1 or 10 μmol/L *S*-(4-nitrobenzyl)-6-thio-inosine (NBTI), 2 mmol/L hypoxanthine, 2 mmol/L adenine, 100 μmol/L 5HT, or 500 μmol/L adenosine, was measured.
Results: Overall adenosine transport (i.e., hENT1+hENT2) was semisaturable and partially inhibited by 1 μmol/L, but abolished by 10 μmol/L NBTI in cells non-treated or treated with NH_4Cl. The initial velocity and non-saturable, lineal component for overall transport were increased after NH_4Cl pulse. hENT1 and hENT2-mediated adenosine transport maximal capacity was increased by acidic pHi. hENT1 activity was more sensitive than hENT2 activity to acidic pHi.
Discussion: hENT1 and hENT2-adenosine transport is differentially regulated by acidic pHi in HUVECs. These findings are important in pathologies associated with pHi alterations such as gestational diabetes mellitus.

1. Introduction

The maintenance of a physiological intracellular (pHi) and extracellular (pHo) pH is under light modulation by plasma membrane transport mechanisms that remove protons (H^+) to the extracellular space [1–3]. These phenomena include the sodium (Na^+)/H^+ exchanger 1 (NHE1) as the primary regulator of the pHi/pHo ratio. Uptake of metabolic substrates happens via different membrane transport systems in adult and foetoplacental endothelium [4,5]. Some of these transport systems depend on the pHo, such as those mediating adenosine and 5-hydroxytryptamine (5HT) [6]. The endogenous nucleoside adenosine is reported to increase the L-arginine transport and synthesis of nitric oxide (NO)

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in the human foetoplacental micro and macrovascular endothelium from normal or pathological pregnancies such as gestational diabetes mellitus (GDM) [7–10]. This phenomenon results from activation of adenosine receptors due to the extracellular accumulation of this nucleoside arising from a reduced uptake by the foetoplacental endothelium [8,10,11]. Thus, a proper function of membrane transport mechanisms modulating the extracellular concentration of adenosine is critical to maintaining the physiological cell metabolism in this vascular bed [4,8–10] as in other tissues [5,6].

Adenosine nucleoside is taken up via the human equilibrative nucleoside transporters (hENTs) in the human umbilical vein (HUVECs) [11] and placental microvascular (hPMECs) [12] endothelial cells. hENTs corresponds to a family of at least four proteins, i.e., hENT1, hENT2, hENT3, and hENT4. hENT1 and hENT2 mediate adenosine uptake in HUVECs from normal or pathological pregnancies, including GDM [11]. Despite the proposed activation of hENT4-mediated adenosine and 5HT transport in response to an acidic pHo in HUVECs [6], the role of a change in pHi or pHo regulating hENT1 and hENT2 transport activity in this or other cell type is unknown [3,5]. One study shows that an acidic pHi increases adenosine efflux in perfused rat skeletal muscle [13], but not an attempt to address the type(s) of membrane transporters involved in this phenomenon was given. Additionally, pre-gestational diabetes mellitus associated with acidic pH (pH < 7.2) in the umbilical vein blood [14]. Since GDM also associates with lower hENT1 and hENT2-mediated adenosine transport in HUVECs [11] and hPMECs [12], we hypothesize that adenosine transport mediated via hENT1 and hENT2 is under regulation by an acidic pHi in HUVECs.

2. Materials and methods

See the expanded Methods section in the online Supplementary material.

2.1. Umbilical cords and cell culture

Human placentas were collected after delivery from 31 full-term normal pregnancies from the Hospital Clínico UC-CHRISTUS in Santiago de Chile (investigation conforms to the Declaration of Helsinki, and counts with Ethics Committee approval from the Faculty of Medicine of the Pontificia Universidad Católica de Chile and informed consent of patients). Sections of umbilical cords were transferred into 200 mL phosphate-buffered saline (PBS) solution ((mmol/L): 130 NaCl, 2.7 KCl, 0.8 Na_2HPO_4, 1.4 KH_2PO_4 (pH 7.4, 4 °C)) to the laboratory.

Human umbilical vein endothelial cells (HUVECs) were isolated by digestion with collagenase from umbilical cord veins and cultured (5% O_2, 5% CO_2, 37 °C) in primary culture medium (PCM: medium 199 (M199, Gibco Life Technologies, Carlsbad, CA, USA), 5 mmol/L D-glucose, 10% new born calf serum, 10% foetal calf serum (Gibco), 3.2 mmol/L L-glutamine, 100 U/mL penicillin-streptomycin (Gibco)) as described [10,15]. Experiments were in primary cultured cells in passage 3 in the absence or presence of 1 or 10 μmol/L *S*-(4-nitrobenzyl)-6-thio-inosine (NBTI) (Sigma, Atlanta, GA, USA),

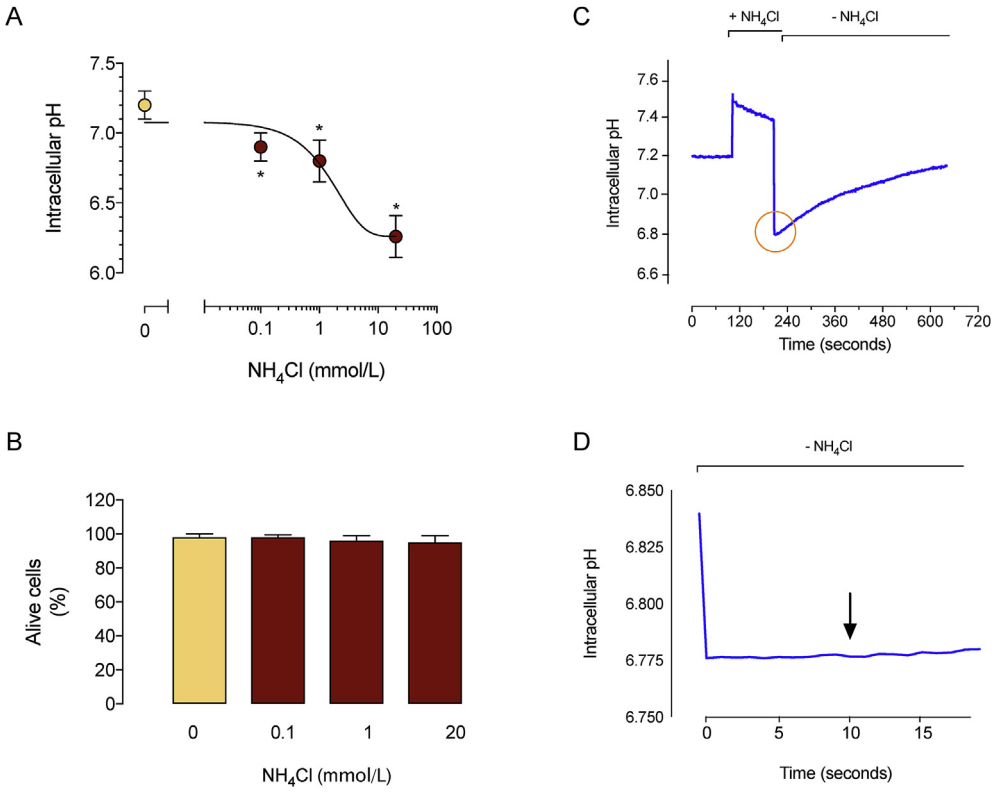

Fig. 1. Effect of NH_4Cl pulse on cell pHi and viability. A, Primary cultures of HUVECs were preloaded with BCECF-AM and transferred into a spectrofluorometer. Basal pHi was stabilized and then cells were exposed (~2 min) to a Na^+-free solution without (0) or with NH_4Cl. Cells were then rinsed with NH_4Cl-free solution and left in this medium. pHi values were estimated from a calibration curve with nigericin (see Methods). B, Alive cells counted in a haemocytometer under the same conditions as in A. C, Cells were preloaded with BCECF-AM and transferred into a spectrofluorometer. After basal pHi was stabilized the cells were exposed (2 min) to a control solution containing 20 mmol/L NH_4Cl (+NH_4Cl). Cells were then rinsed with NH_4Cl-free solution (− NH_4Cl) and left in this medium. pHi values were estimated as in A. The circle indicates the section of the trace shown in D. D, Data for the first 15 s after removal of NH_4Cl as in C. Arrow indicates the time (10 s) used for transport assays. In A and B, $^*P < 0.05$ versus without NH_4Cl. Values are mean ± S.E.M. (n = 22). In C and D, Data is representative of other 27 different cell cultures.