Modelling the Impact of Astrocytic NBCe1 on Ischemia-Induced Astrocytic Na^+ Loading and ATP Depletion in Mouse Neocortex

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Abstract

Ischemia leads to increased sodium concentration in astrocytes, disrupting ionic balance and causing cellular damage. Astrocytes have high levels of NBCe1, an electrogenic sodium-bicarbonate cotransporter that regulates intracellular pH and operates near its reversal potential. We investigated how NBCe1 functions during transient energy deprivation using mathematical simulation of astrocytic pH, sodium (Na^+), and ATP in mouse neocortical slices. Metabolic inhibition to mimic ischemic conditions caused temporary acidosis, increased Na^+ levels, and decreased ATP levels in astrocytes. Blocking NBCe1 intensified astrocytic acidosis during ischemia, while reducing Na^+ accumulation and ATP loss. Similar results were observed in NBCe1-deficient mice compared to wild-type. Fluorescence imaging confirmed these findings. In conclusion, our data demonstrate that transient energy failure activates NBCe1 inwardly in astrocytes, mitigating astrocytic acidosis during ischemia but leading to increased Na+ influx and decreased cellular ATP.

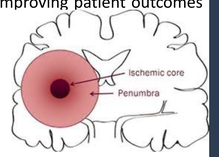
Introduction

Ischemic stroke remains a leading cause of mortality and morbidity worldwide, necessitating a deeper understanding of its underlying mechanisms for effective therapeutic interventions. Central to the pathophysiology of ischemic stroke are the concepts of the "ischemic core" and the "ischemic penumbra," which represent distinct regions within the infarcted brain tissue. The ischemic core experiences severe and irreversible damage due to insufficient blood flow, leading to rapid ATP depletion and neuronal cell death. In contrast, the ischemic penumbra, the border zone surrounding the core, offers a critical window of opportunity for intervention as its cells are partially compromised but potentially salvageable.

Astrocytes, the most abundant glial cell type in the central nervous system, play a pivotal role in maintaining brain homeostasis. Among their numerous functions, astrocytes are essential for regulating extracellular ion concentrations and providing energy substrates, primarily in the form of ATP, to neurons. Importantly, during ischemic events, astrocytes can either exacerbate or mitigate the damage occurring in the penumbra and core regions.

One key molecular player in this complex interplay is the sodium-bicarbonate cotransporter isoform 1 (NBCe1), predominantly expressed in astrocytes. NBCe1 is responsible for the transport of sodium (Na^+) and bicarbonate (HCO_3^-) ions across the cell membrane, thereby influencing intracellular pH and Na^+ homeostasis. Dysregulation of NBCe1 activity has been linked to altered Na^+ levels in astrocytes, which can, in turn, impact their capacity to support neighboring neurons during ischemia [1, 2].

Understanding the role of astrocytic NBCe1 in modulating Na^+ loading and ATP depletion during ischemia is of paramount importance, as it may shed light on potential therapeutic targets to protect and salvage neurons within the ischemic penumbra. In this study, we extend the model describe in [3, 4] to simulate the dynamics of astrocytic NBCe1 activity and its effects on Na^+ homeostasis and ATP availability in the mouse neocortex during ischemic events. Our model successfully reproduce the experimental events for two minutes chemical ischemia. We then predict the role of potassium for NBCe1 reversibility during severe Na^+/K^+-ATPase (NKA) inhibition. By elucidating these intricate mechanisms, we aim to contribute to the development of innovative strategies aimed at preserving brain function and improving patient outcomes in the aftermath of ischemic stroke.



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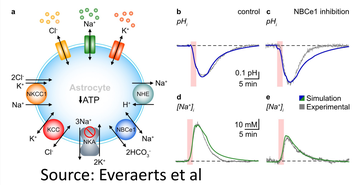
Methodology

Model pathways and equations



Source: Everaerts et al under-review cells

Computational modelling of ischemia-induced pH_i and $[Na^+]_i$ changes in astrocytes. (a) Schematic of the main pathways incorporated in the model. The arrow heads represent the direction of the flux. (b) Illustrating ischemia-induced pH_i and $[Na^+]_i$ changes in control (left panel) and upon inhibition of NBCe1 (right panel) in experimental and simulated conditions over a time course of 30 minutes. The blue and green traces represent simulated changes, the grey traces are experimental data taken from Fig. 2a, b. Periods of chemical ischemia are indicated by the light red areas.

pH dynamics

$$\frac{dpH_i}{dt} = \frac{J_{NHE} + J_{NBCe1}}{\beta_{tot}},$$

$$\beta_{tot} = \beta_{i/o} + \frac{2.3[HCO_3^-]_{i/o}}{pH_{rest_{i/o}}}$$

Sodium dynamics

$$\frac{d[Na^+]_i}{dt} = -J_{Na} - 3J_{NKA} + J_{NKCC1} + J_{NBCe1} - J_{NHE}$$

$J_{NKA} = J_{NKA_{max}}\,(-I_1\,(a_1, b_1, t, t_0, c_1)\,I_2\,(a_2, b_2, t, t_0, c_2) + d)\,H_{1.5}([Na^+]_i, K_{Na_i})\,H([K^+]_o, K_{K_o})$ where J_{NKAmax} is the maximum flux through Na^+/K^+–ATPase and $H_n(x,K)$ is of the form $\frac{x^n}{x^n + K^n}$, where n is the Hill coefficient, x is the concentration of Na^+ or K^+, and K in the function $H_n(x,K)$ is the dissociation constant of the respective ion to the pump $I(a, b, t, t_0, c) = \frac{a}{1 + a\exp(b(t - t_0) + c)}$

where a, b, c, and d are constants, t represents time during the simulation, and t_0 represents the time at which ischemia is initiated. Function I mimic reduced oxygen and blood flow in brain.

Numerical Method:

The rate equations are solved in Fortran 90 using the Euler method with a time step of 0.1 µs. The system of equations is allowed to reach steady state before imposing chemical ischemia. Data are visualized using MATLAB.

Experiment

Preparation of Organotypic Slice Cultures:

- Used wild-type and NBCe1 KO mice (P6-9).
- Slices (250 µm) prepared from mouse brains.
- Cultured in MEM-based medium at 36°C (5% CO2/95% O2).
- Medium replaced every 3 days.
- Experiments on slices aged 10-21 days.

Imaging of Intracellular pH and $[Na^+]$:

- Used BCECF and SBFI for pH_i and $[Na^+]_i$ measurements.
- BCECF: Excitation at 458 nm and 488 nm.
- SBFI: Excitation at 400 nm, emission above 430 nm.
- Calibration for accurate mM ($[Na^+]$) and pH unit conversion.
- Calibration covers baseline $[Na^+]_i$ (12.1 ± 0.5 mM) and pH_i (7.33 ± 0.04).

FRET-based Imaging of Intracellular ATP:

- Employed ATeam1.03YEMK (ATeam) sensor.
- Transduced slices with ATeam-expressing vector (AAV 2/5).
- Excited ATeam at 434 nm.
- Measured fluorescence ratio (Venus/eCFP) in astrocytic cell bodies.

Measurement of Extracellular $[K^+]$, pH, and $[Na^+]$:

- Used double-barreled ion-sensitive microelectrodes.
- Calibrated electrodes before and after each experiment.
- K^+-sensitive electrodes calibrated in HEPES-based saline.
- Na^+-sensitive electrodes calibrated with Na+ solutions.
- pH-sensitive electrodes calibrated with pH 7.0 and 7.6 salines.

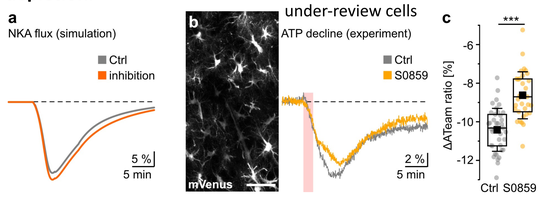
Patch-Clamp Recordings:

- Monitored astrocytic membrane potential with cell-attached patch-clamp recordings.
- Used patch pipettes (3.5-4.0 MΩ) filled with standard aCSF.
- Conducted electrophysiological measurements with EPC10 amplifier.

Results

Role of NBCe1 in ischemia-induced astrocytic ATP depletion.

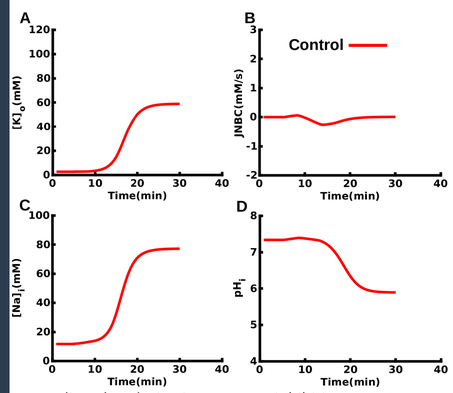
Source: Everaerts et al under-review cells



In (a), computational modeling indicates reduced NKA flux in astrocytes upon NBCe1 inhibition, leading to decreased ATP consumption. (b) ATeam fluorescence shows changes during chemical ischemia induction in control and with NBC inhibition. (c) Experimental results are summarized in box plots, with significant differences ($p < 0.001$) between groups. Transient Ischemia induced for 2 minutes

Role of extracellular potassium (Ko) in NBCe1 reversal during intense NKA inhibition. Computational modeling of 80% NKA inhibition for 20 minutes.

Prediction



(A) Extracellular K^+ rises due to ATP loss. (B) NBC flux with distinct inward, reverse and depolarized phase. (C) Intracellular Na^+ load resembling core. (D) Intense decrease in intracellular pH.

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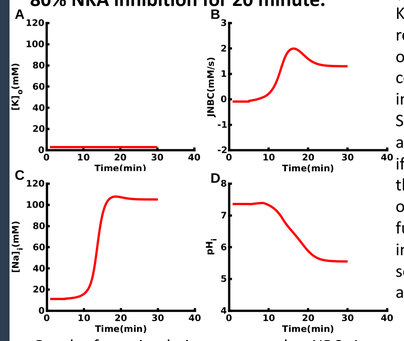
predicts that during intense NKA inhibition NBCe1 reverse. Three phases of NBCe1 activities were observed. First phase is due to extracellular pHo, reversal phase is due to Ko and third phase is due to Na^+ load and depolarization due to Ko. The high Na^+ load signify the severe depletion of ATP followed by strong acidification

Effects of resting Ko in NBCe1 during 80% NKA inhibition for 20 minute.



(A) At resting extracellular K^+. (B) NBC flux representing inward operation of NBCe1 cotransporter. (C) Intense intracellular Na^+ load. (D) Strong intracellular acidification. It suggest that if Ko does not change, there occurs inward operation of NBCe1. This further accumulate the intracellular Na^+ suggesting severe depletion of ATP and strong acidification.

Result of our simulations suggest that NBCe1 reversibility is dependent upon the extracellular potassium change.

Discussion/Conclusion

1.NBCe1 Activity during Brief Chemical Ischemia: NBCe1 operates in an inward mode during brief chemical ischemia. This suggests that it is involved in the uptake of bicarbonate ions into cells in response to ischemic conditions.

2.ATP Depletion and NBCe1: NBCe1 activation is associated with higher ATP depletion compared to inhibition. This implies that the activity of NBCe1 requires energy in the form of ATP and that it is more energy-consuming when it is activated.

3.NBCe1 Operating in Reverse Mode: If the intensity of the ischemia is high, NBCe1 could operate in reverse mode. This means that under different conditions, NBCe1 may switch its function and transport bicarbonate ions out of the cell.

4.Potassium's Role in NBCe1 Reversal: Potassium could play an important role in membrane depolarization and NBCe1 reversal. Potassium ions are crucial for maintaining the membrane potential, and alterations in potassium levels could affect the direction of ion transport by NBCe1.

5.Intense Ischemia Effects: In intense ischemia, several significant changes occur:

1. Higher Sodium (Na^+) levels: This suggests increased sodium influx, which could be a consequence of NBCe1 operating in reverse mode or other ion transporters being affected.
2. Strong Depolarization: The strong depolarization of the cell membrane indicates a disruption of the normal ion gradients, likely due to changes in ion transport processes, including NBCe1.
3. Intense Acidification: Acidification can result from increased intracellular levels of protons (H^+ ions) due to altered ion transport. NBCe1 may contribute to this acidification by transporting bicarbonate ions.

In conclusion, these points suggest that NBCe1 is a transporter protein that plays a role in regulating bicarbonate ion transport in response to ischemic conditions. Its activity is influenced by the severity of ischemia, and it can switch between inward and reverse modes. Potassium and other ion transport processes are likely interconnected with NBCe1 activity during ischemia, leading to changes in intracellular ion concentrations, membrane potential, and acidification. Further research is needed to fully understand the intricate mechanisms and consequences of NBCe1 function in ischemic conditions.

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Please use the following link for the supplementary Information:
https://github.com/pawanthapaliya01/SFN_conference_file.git

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