Investigation of GABA transport and the GABA/Na⁺ relationship in human GAT1 using solid supported membrane-based electrophysiology

Rocco Zerlotti[1], Andre Bazzone[1], Maria Barthmes[1], Nina Brinkwirth[1], Cecilia George[1], Niels Fertig[1]

1 Nanion Technologies, GmbH, Germany

Contact: Rocco.Zerlotti@nanion.de

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1 Introduction

The primary regulator of γ-aminobutyric acid (GABA) homeostasis in the central nervous system is the human GABA transporter 1 (hGAT1). Functioning as a secondary active transporter, hGAT1 utilizes the inward-directed Na^+ electrochemical gradient to facilitate the uphill reuptake of GABA from the synaptic cleft to the presynaptic neuron. The apparent affinity (K_M) for GABA reuptake is established to be in the low µM range (Bicho, 2005; Gonzales, 2007), and the widely accepted stoichiometry of GABA transport is currently understood as Na^+:Cl^-:GABA = 2:1:1.

In this study, CHO plasma membrane vesicles with stable hGAT1 overexpression were utilized to measure GABA-induced currents through solid supported membrane-based electrophysiology (SSME). This technique also allowed for an examination of the cooperativity between Na^+ and GABA and its effect on transport currents. Furthermore, the GABA:Na^+ stoichiometry was evaluated in the course of the investigation.

2 SSME – an overview

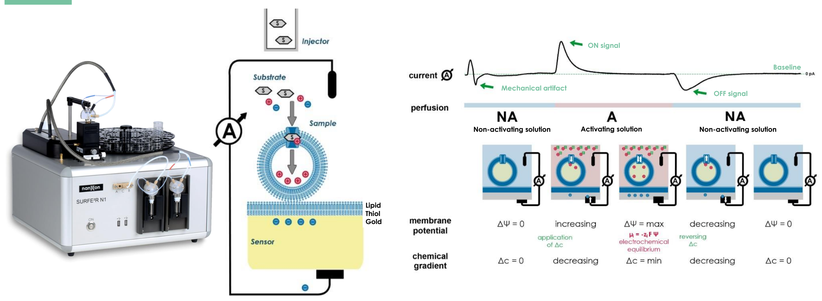
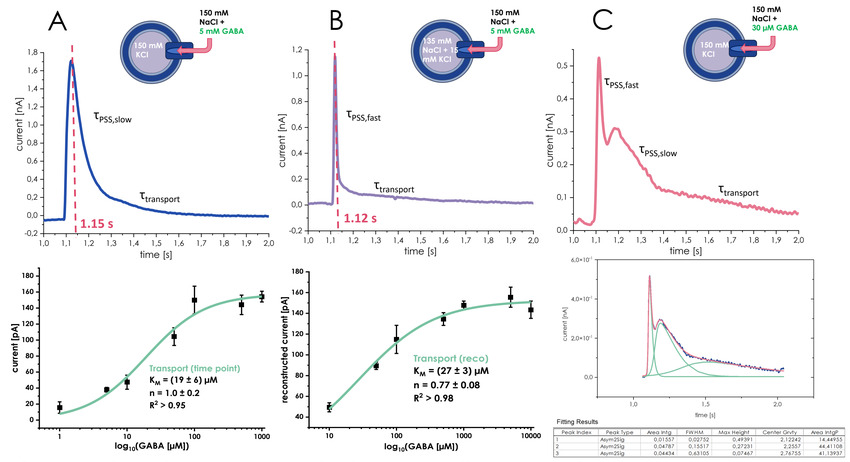


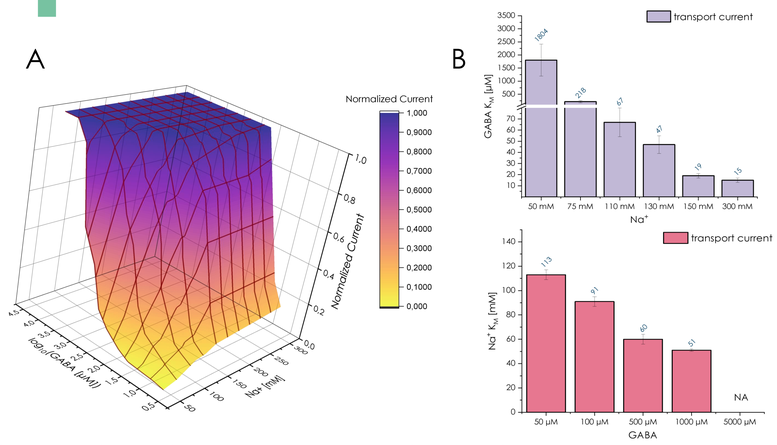
Diagram of the measurement chamber in a SURFE2R N1 and the measurement principle of SSME. The protein of interest (expressed in membrane vesicles or reconstituted in proteoliposomes) adheres to an artificial bilayer on top of a gold-coated sensor. The measurement occurs at 0 mV, and the introduction of substrate creates a concentration gradient that propels the transport reaction. In a typical SSME workflow, a non-activating solution is perfused to establish the electrical signal baseline and, if necessary, to generate a co-substrate gradient for transport activation. Subsequently, the activating solution with the primary substrate is perfused, initiating transport. Due to the capacitive-coupled nature of the system, only transient currents are recorded. Finally, a second flow of non-activating solution restores the system to its initial state.

3 GABA-induced currents and GABA K_M with SSME



A) Above: GABA-induced currents in high Na^+ gradient; Below: GABA K_M in high Na^+ gradient
B) Above: GABA-induced currents in low Na^+ gradient; Below: GABA K_M in low Na^+ gradient
C) Above: GABA-induced currents at low GABA; Below: peak deconvolution

4 GABA/Na+ cooperativity



A) 3-D plot of normalized transport current as a function of both GABA and Na^+ concentrations. **B)** Above: GABA K_M as a function of Na^+ concentrations. Notably, the K_M starts increasing by 10-factors when Na^+ concentrations drop below 100 mM. Below: Na^+ K_M as a function of GABA concentrations. In this case lowering GABA from 1 mM to 50 µM only affects the Na^+ K_M by a factor 2.

5 GABA:Na+ stoichiometry with SSME

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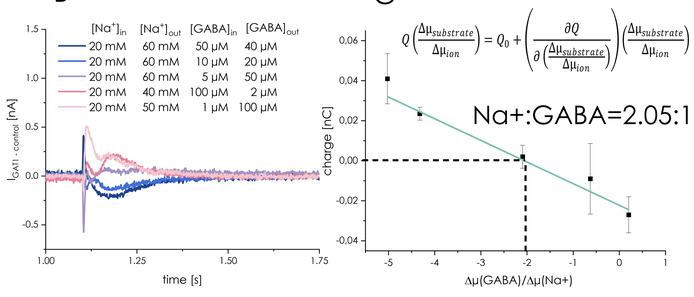
Since the measurement is generated at 0 mV, the Gibbs free energy (ΔG) can be re-written as a linear combination of the chemical potentials ($\Delta\mu$) multiplied by the stoichiometric coefficients (m, n):

$$\Delta G = m\Delta\mu_{GABA} + n\Delta\mu_{Na^+}$$

When the total charge (Q) is 0, the ΔG is also 0 and the equation above can be rewritten as:

$$\frac{\Delta\mu_{GABA}}{\Delta\mu_{Na^+}} = \frac{ln\left(\frac{[GABA]_{in}}{[GABA]_{out}}\right)}{ln\left(\frac{[Na^+]_{out}}{[Na^+]_{in}}\right)} = -\frac{n}{m}$$



A) Physical principle of stoichiometric assay with SSME. **B)** Traces obtained at all different conditions of Na^+ and GABA inside and outside the vesicles. The resulting traces from CHO vesicles expressing GAT1 have been compared with traces from empty CHO vesicles used as control, which have then been subtracted from the sample traces. **C)** Resulting linear fitting of the data. When the total charge is null, then the ratio between the chemical potential of GABA and Na^+ reflects the stoichiometry of the two substrates in the transport reaction (Thomas, 2021). At 0 charge, the stoichiometric ratio reflects a stoichiometry GABA:Na^+=1:2.

6 Summary

- SSME is a suitable technique for studying hGAT1, revealing multiple electrogenic events upon GABA binding and transport.
- The cooperativity between GABA and Na^+ has been investigated, showing that Na^+ concentrations greatly affect GABA K_M.
- The stoichiometry of Na^+ and GABA has been successfully assessed with SSME, confirming the most accepted one of GABA:Na^+=1:2.

Bibliography:

- Bicho et al., "*Rapid Substrate-Induced Charge Movements of the GABA transporter GAT1*" DOI: https://doi.org/10.1016/j.jbc.2021.101220
- Gonzales et al., "*Turnover rate of the gamma-aminobutyric acid transporter GAT1*" DOI: 10.1007/s00232-007-9073-5
- Thomas et al., "*A solid-supported membrane electrophysiology assay for efficient characterization of ion-coupled transport*" DOI: https://doi.org/10.1016/j.jbc.2021.101220