Functional characterization of human GAT-1 using solid supported membrane electrophysiology and automated patch clamp

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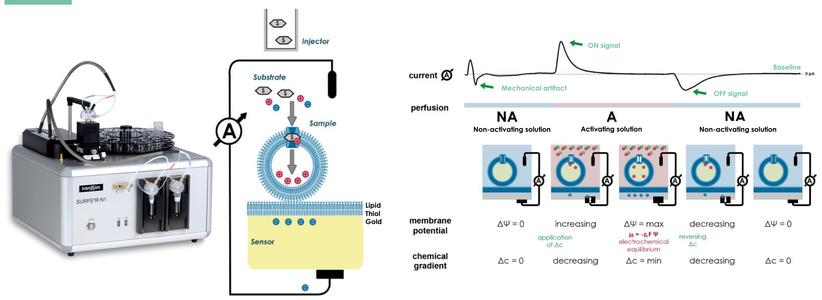
1 Introduction

The human γ-aminobutyric acid (GABA) transporter 1 (hGAT1) is the main regulator of GABA homeostasis in the central nervous system. It is a secondary active transporter that exploits the inward-directed Na^+ chemical gradient to energize the uphill re-uptake of GABA from the synaptic cleft to the presynaptic neuron.

The apparent affinity (K_M) for GABA re-uptake is known to be in the low µM range and the currently most accepted stoichiometry of GABA transport is Na^+:Cl^-:GABA = 2:1:1.

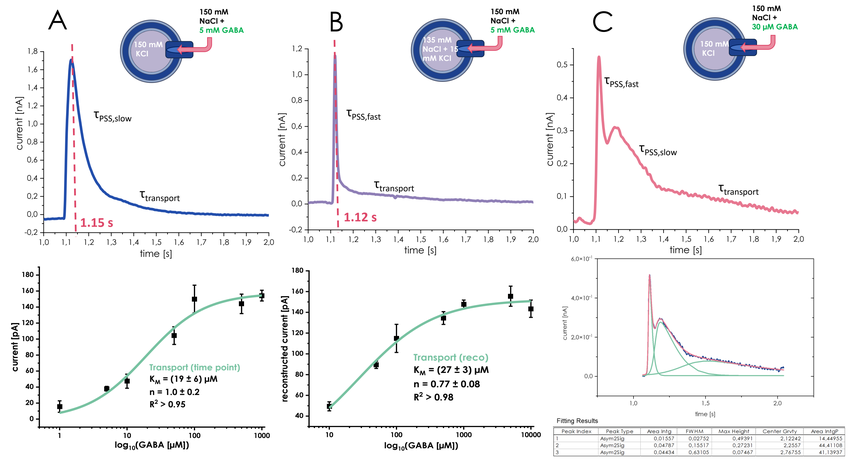
Here, CHO plasma membrane vesicles stably overexpressing hGAT1 were used to measure GABA induced currents and GABA:Na^+ stoichiometry with solid supported membrane-based electrophysiology (SSME) using a SURFE^{2}R N1 system. We also measured GABA transport through automated patch clamp using a Patchliner, confronting the GABA concentration dependence with literature results.

2 SSME – an overview



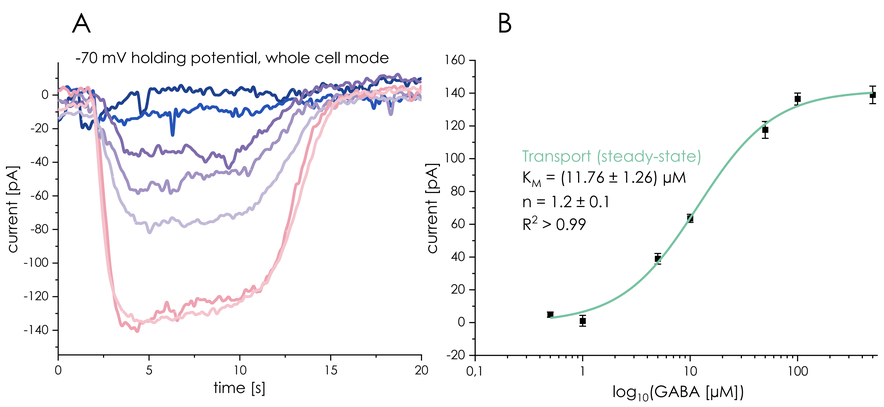
Schematic of the measurement chamber of a SURFE^{2}R N1 and measurement principle of SSME. The protein of interest (expressed in membrane vesicles or reconstituted in proteoliposomes) is adsorbed to an artificial bilayer that functionalize a gold-coated sensor. The measurement is carried out at 0 mV and the addition of substrate generates a concentration gradient that drives the transport reaction. In a general SSME workflow a non-activating solution is perfused to obtain the electrical signal baseline and – if needed, to generate a co-substrate gradient to energize transport. Then, the activating solution containing the main substrate is perfused and transport is activated: because of the capacitive-coupled nature of the system, only transient currents are recorded. Finally, a second flow of non-activating solution brings the system back to the initial state.

3 GABA-induced currents and GABA K_M with SSME



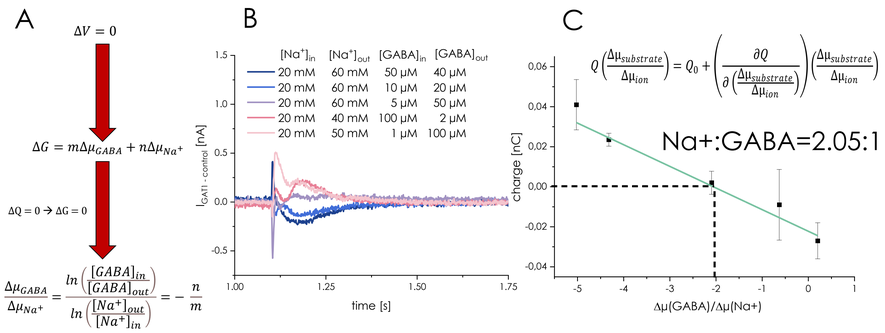
A) Above: GABA-induced currents in high Na^+ gradient; Below: GABA KM in high Na^+ gradient
B) Above: GABA-induced currents in low Na^+ gradient; Below: GABA KM in low Na^+ gradient
C) Above: GABA-induced currents at low GABA; Below: peak deconvolution

4 GABA-induced currents and GABA K_M with patch clamp



A) Traces obtained with CHO cells over-expressing hGAT1, using a Patchliner in whole cell mode and at membrane potential of -70 mV. Solutions containing GABA and NaCl have been perfused for 10 seconds. **B)** GABA K_M obtained with the Patchliner. The result is in perfect agreement with literature data (Bicho, 2005; Gonzales, 2007) and in very good agreement with SSME data.

5 GABA:Na^+ stoichiometry with SSME



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 cells from which the vesicles for SSME have been generated have been tested with patch clamp, validating the functionality of the sample.
- 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