Application Note

Transporter:	hGAT1
Sample:	Plasma membrane
Origin:	HEK cells
Tools:	SURFE²R N1

Electrophysiological recordings of hGAT1 (SLC6A1) activity on Nanion's SURFE²R N1

The electrophysiology team at Nanion Technologies GmbH, Munich.
Cells kindly provided by Axxam S.p.A

Summary

GABA is the major inhibitory neurotransmitter in the brain and is important in controlling excitability. After release, GABA is removed from the extracellular space by GABA transporters (GATs), thus terminating inhibitory synaptic transmission. The GABA transporters belong to the family of neurotransmitter:sodium symporters referred to as the solute carrier 6 (SLC6) family in humans[1]. GATs co-transport GABA, Na^+ and Cl^- with the proposed stiochiometry 1 GABA: 2 Na^+: 1 Cl^-, resulting in a net influx of 1 positive charge per cycle[2]. So far, 4 GATs have been identified, GAT1, GAT2, GAT3 and BGT1[3].

GAT1 is expressed throughout the brain in both GABAergic and non-GABAergic neurons, and is expressed in particularly high levels in the olfactory bulb, basal ganglia, cerebellum and retina[4]. The physiological function of GAT1 is primarily to terminate synaptic transmission but also to ensure the fidelity of synaptic transmission by preventing the spread of neurotransmitter to neighbouring synapses[5]. GABA transporters also play an important role in neurotransmitter reutilization[5]. In certain circumstances, e.g. when the sodium gradient increases during ischemia or following seizures, GATs can act in reverse which may have a protective effect during seizures, by inhibiting electrical excitability[5]. There is some evidence that GATs may play a role in neurodegenerative diseases such as Parkinson's[3] and Alzheimer's[6] and may provide a novel target for treating these conditions.

Here we present human GAT1 activity measurements on the SURFE²R N1 instrument using purified plasma membranes from HEK cells. GABA affinity and effect of inhibitors were investigated.

Download more Application Notes from www.nanion.de

Results

To activate transport on the SURFE²R N1, a sensor with attached hGAT1-containing plasma membranes was inserted into the device and perfused with a buffer containing NaCl and GABA. When the substrate is present, Na^+ and Cl^- movement across the membrane can be observed until an electrochemical equilibrium is reached. To generate Na^+ and Cl^- gradients, necessary as a driving force, the sensor was flushed with Kgluconate to create a Na^+ and Cl^- gradient simultaneously before and after the hGAT1 activation (Figure 1). To ensure stable GAT1 current response for multiple activation cycles, a waiting time and thorough rinsing of the sensor was necessary between measurements.

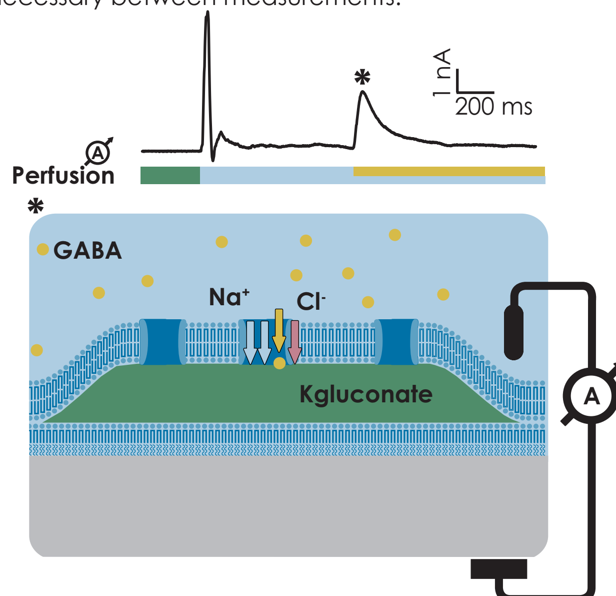

Figure 1: Typical GAT1 current response on the SURFE²R N1. GABA was used as the substrate for GAT1. When applying GABA to the sensor, a transient current occurs (top) corresponding to the co-transport of GABA, Na^+ and Cl^- (bottom).

nan]i[on

Application Note

The affinity of GAT1 for GABA was investigated. GABA was applied in increasing concentrations giving an apparent affinity of 0.95 ± 0.10 mM (Figure 2).

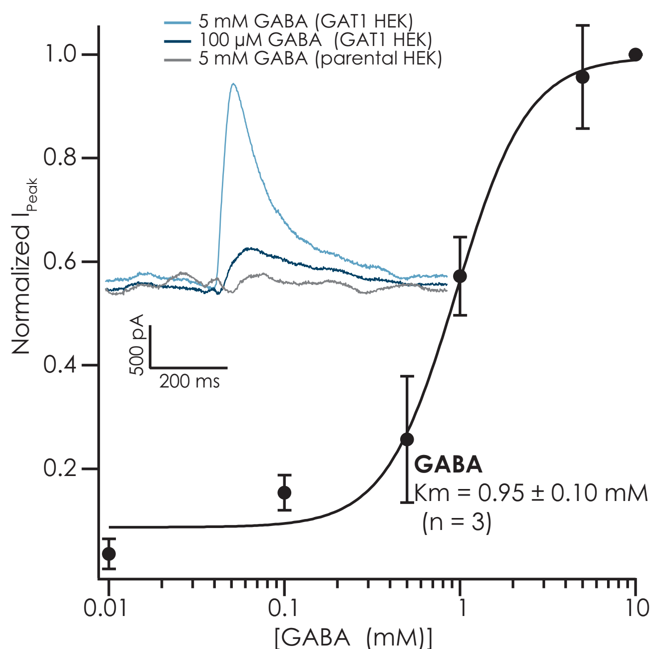

Figure 2: Affinity of GAT1 for GABA. GABA was applied in increasing concentrations revealing a Km = 0.95 ± 0.10 mM (n = 3). In cells not expressing GAT1 (parental HEK), no transport was observed (grey).

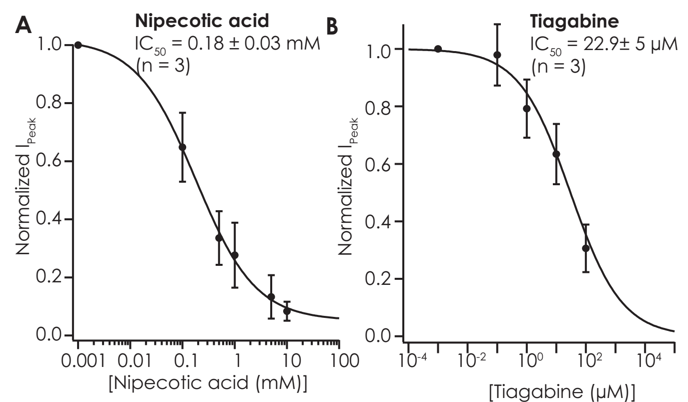

Figure 3:
GAT1 is blocked by nipecotic acid (A) and Tiagabine (B). Experiments performed in the presence of 1 mM GABA.

GAT1 was blocked by nipecotic acid and tiagabine with an IC_{50} of 0.18 ± 0.03 mM (n = 3) and 22.9 ± 5 µM (n = 3), respectively. These values are a little higher than those found in literature[5,7], presumably due to differences in technique used.

In conclusion, the SURFE²R N1 can be used to reliably measure GAT1 activity using whole cells attached to the sensors. The assay could be scaled up to the SURFE²R 96SE for higher throughput screening and hit identification for GAT1 inhibitors for the treatment of neurodegenerative diseases such as Parkinson's and Alzheimer's.

References

1. Scimemi, A. 2014. Front. Cell. Neurosci. 8:161.
2. Loo, D.D.F., *et al*. 2000. J. Biol. Chem. 275(48): 37414–37422
3. Jin, X-T. *et al*. 2011. Front. Sys. Neurosci. 5: 63
4. Durkin, M.M., *et al*. 1995. Mol. Brain Res. 33: 7-21
5. Borden, L.A. Neurochem. Int. 1996. 29(4): 335–356
6. Fuhrer, T.E., *et al.* Neurosci. 2017. 351:108-118
7. Soudijn, W. & van Wijngaarden, I. 2000. Cur. Med. Chem. 7: 1063-1079

Methods

Plasma membrane preparation

HEK cells expressing hGAT1 were kindly provided by Axxam S.p.A and membranes were prepared according to the Nanion's standard procedure ("Quickguide Membrane Preparation from CHO cells"). Total protein concentration was between 5 - 10 µg/µl.

Buffers

GAT1 experiments were performed by the exchange of a sodium and GABA-free ("resting") buffer for a sodium containing ("control") buffer and afterwards a substrate and sodium containing ("activating") buffer. Resting buffer contained: 140 mM Kgluconate, 2 mM $MgCl_2$ 30 mM HEPES, pH 7.4 with NMG. Control buffer contained: 140 mM NaCl, 2 mM $MgCl_2$ 30 mM HEPES, pH 7.4 with NMG, activating buffer identical to control plus x mM GABA.

SURFE²R sensor preparation

According to the Nanion standard procedure "SURFE2R Sensor Preparation". Sensors are prepared in resting buffer, membrane is diluted 1:10 with resting buffer.

SURFE²R N1 measurement workflow

GAT1 can be activated by providing GABA as the substrate. A sodium gradient needs to be established in advance of substrate addition. Therefore, any 3-buffer Nanion standard protocol is suitable.

Nanion Technologies GmbH
Ganghoferstr. 70A
80339 Munich, Germany

phone +49 89 219 095-0
fax +49 89 218997960
www.nanion.de • info@nanion.de

nan]i[on