Application Note

Channel: CNT1
Cells: CHO
Tools: SURFE²R N1

Electrophysiological recordings of CNT1 (SLC28A1) activity on Nanion's SURFE²R N1

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Summary

The concentrative nucleoside transporter 1 (CNT1) is a sodium-dependent uptake transporter encoded by the SLC28A1 gene[1]. CNT1 functions as a co-transporter, coupling the uphill nucleoside transport into the cells to the electrochemical gradient of sodium[2]. The stoichiometry of transport is proposed to be 1:1[3], but a stoichiometry of 2 Na^+: 1 nucleoside has also been suggested[4]. CNT1 is an electrogenic transporter, generating a net charge flow. It plays a major role in the uptake of pyrimidines, including uridine and cytidine, from the extracellular milieu into the cytoplasm[1] in nucleoside salvage pathways which is the first step of nucleoside biosynthesis[2]. The transporter is expressed in epithelial tissues including liver, kidney and small intestine where it is localized to the apical membrane[2]. CNTs are important targets for many antiviral and anticancer agents[5,6], and CNT1 has been proposed to play a role in tumor biology via a mechanism beyond nucleoside transport[7]. In fact, tumors expressing high levels of CNT1 can indicate a higher risk of relapse for breast cancer patients[8], suggesting that nucleoside salvage may interfere with chemosensitivity[8]. On the other hand, high expression of the CNT1 protein could promote drug-induced cytotoxicity if patients were treated with suitable hCNT substrates[1]. In any case, hCNT1 is an important mediator in the transport of anticancer and antiviral nucleoside drugs[1,3,5,6] by mechanisms that require further study.

Here we present CNT1 activity measurements on the SURFE²R N1 instrument using purified plasma membrane of CHO cells expressing CNT1.

Results

To activate CNT1 on the SURFE²R N1, a sensor with attached CNT1-containing membrane fragments was inserted into the device and perfused with a buffer containing NaCl and uridine. When the nucleoside is present, sodium movement across the membrane can be observed until an electrochemical equilibrium is reached. To generate sodium gradients, necessary as a driving force, the sensor was flushed with KCl before and after activation of CNT1 (Figure 1). Uridine elicits a larger response of CNT1 compared with cytidine but CNT1 has a higher apparent affinity for cytidine vs uridine (Figure 2).

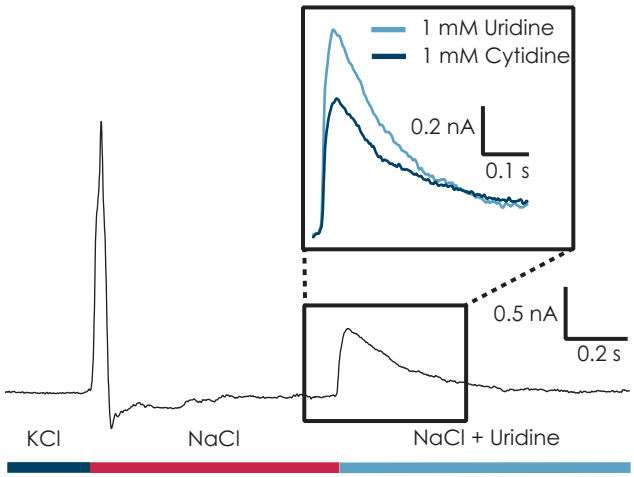

Figure 1: Typical CNT1 current response on the SURFE2R N1. Uridine was used as a substrate for CNT1. After establishing a sodium gradient (first peak), uridine was applied to the sensor (second peak). Uridine or cytidine can be used as the substrate, uridine eliciting a larger response than cytidine (inset).

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The apparent affinity of CNT1 to the substrates uridine and cytidine was investigated. Apparent K_m values of 78.67 ± 21.16 µM (n = 6) for uridine, and 8.24 ± 1.38 µM (n = 5) for cytidine were determined (Figure 2) in good agreement with range found in the literature[3,4,9,10].

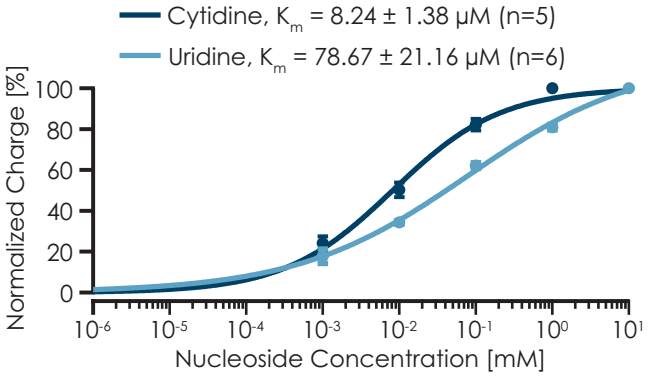

Figure 2: Increasing concentrations of 2 different substrates were added cumulatively on the same population of membrane fragments and the average concentration response curves for cytidine (n = 5) and uridine (n = 6) were constructed. When fitted with a Hill equation, apparent K_m values of 8.24 ± 1.38 µM (n = 5) and 78.67 ± 21.16 µM (n = 6) for cytidine and uridine, respectively, were obtained.

The effect of different ion gradients on CNT1 activity was also investigated. Figure 3 shows charge values for CNT1 under different ionic conditions: Na^+, K^+,

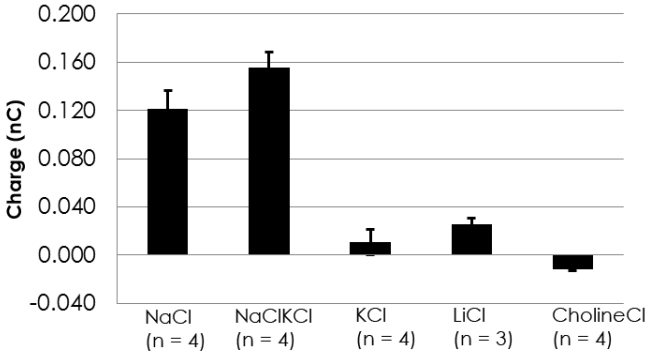

Figure 3: CNT1 co-transports Na^+, Li^+ acts as a weak substrate, K^+ and choline cannot be co-transported by CNT1. A Na^+ gradient (NaClKCl) increases the signal amplitude.

Li^+ and choline were used as the co-transported ion in symmetrical conditions. Significant ionic transfer by CNT1 only occurs in the presence of Na^+, demonstrating strict coupling of nucleotide and sodium transport and a high Na^+ specificity of the transporter. Application of a sodium gradient (NaClKCl) increases the current amplitude, illustrating the resulting increase of driving force.

In conclusion, the SURFE²R N1 can be used to reliably measure CNT1 activity. This has important implications for drug discovery targeting CNT1 because nucleoside analogues are used as anticancer and antiviral therapies.

References

1. Pastor-Anglada, M., *et al.*, 2008. Xenobiotica. 38(7-8):972-994

2. Gray, J.H., *et al.*, 2004. Pflugers Arch. 447:728-734

3. Smith, K.M., *et al.*, 2004. J. Physiol. 558.3:807–823

4. Larráyoz, I.M., *et al.*, 2004. JBC. 279(10): 8999–9007

5. Pastor-Anglada, M., *et al.*, 1998. TiPS. 19:424-430

6. Pastor-Anglada, M., *et al.*, 2005. Vir. Res. 107:151-164

7. Pérez-Torras, S., et al., 2013. Cell Death and Disease 4, e648; doi:10.1038/cddis.2013.173

8. Gloeckner-Hofmann, K., 2006. Oncology. 70:238-244

9. Graham, K.A., *et al.*, 2000. Nucleosides Nucleotides Nucleic Acids. 19(1-2):415-34

10. Griffith, D.A., & Jarvis, S.M. 1996. Biochimica et Biophysica Acta 1286:153-181

Methods

Plasma membrane preparation

According to the Nanion's standard procedure ("Quickguide Membrane Preparation from CHO cells"). Total protein concentration was between 5 - 10 µg/µl.

Buffers

CNT1 experiments were performed by the exchange of a sodium and nucleoside free ("resting") buffer for a sodium containing ("control") buffer and afterwards a nucleoside and sodium containing ("activating") buffer.
Resting buffer contained: 140 mM KCl, 5 mM $MgCl_2$, 30 mM HEPES, pH 7.4 with NMG.
Activating buffer contained: 140 mM NaCl, 5 mM $MgCl_2$, 30 mM HEPES, pH 7.4 with NMG, x mM uridine/cytidine.

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

CNT1 can be activated by providing uridine and cytidine as a nucleoside. A sodium gradient must be established in advance of nucleoside addition. Therefore, any 3-buffer Nanion standard protocol is suitable.

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