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 cytoplasm1 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 druginduced cytotoxicity if patients were treated with suitable hCNT substrates1. 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 CH 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).

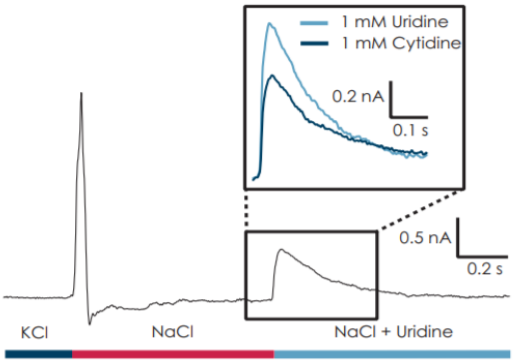

Figure 1: *Typical CNT1 current response on the SURFE2R N1. Uridine was used as a substrate for CNT1. After establishing a sodium gradient (first peak), uridine was applied to the sensor (second peak). Uridine or cytidine can be used as the substrate, uridine eliciting a larger response than cytidine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

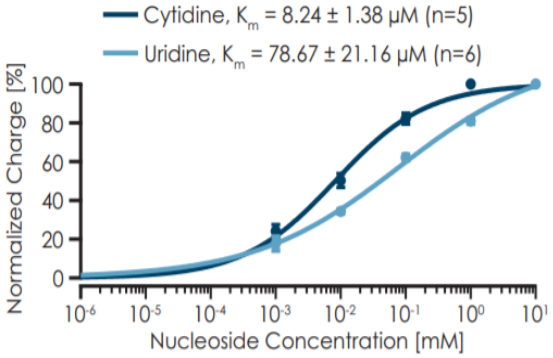

Figure 2: *Increasing concentrations of 2 different substrates were added cumulatively on the same population of membrane fragments and the average concentration response curves for cytidine (n = 5) and uridine (n = 6) were constructed. When fitted with a Hill equation, apparent Km values of 8.24 ± 1.38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

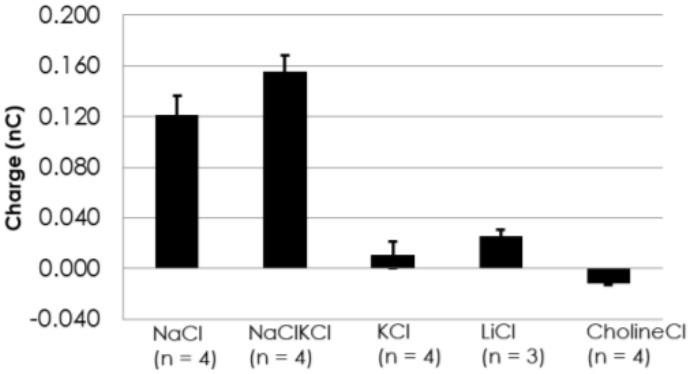

Figure 3: *CNT1 co-transports Na^+, Li^+ acts as a weak substrate, K^+ and choline cannot be co-transported by CNT1. A Na^+ gradient (NaClKCl) increases the signal amplitude.*

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

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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 MgCl2, 30mM 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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