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Short Communication

EXPRESSION OF A HUMAN RENAL SODIUM NUCLEOSIDE COTRANSPORTER IN *XENOPUS LAEVIS* OOCYTES

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Abstract—In this study, *Xenopus laevis* oocytes injected with poly(A)$^+$ RNA (mRNA) isolated from human kidney were used to express a Na$^+$-nucleoside cotransporter. Na$^+$-stimulated [^{3}H]thymidine uptake was enhanced 2–3-fold in oocytes injected with 50 ng poly(A)$^+$ RNA and 4–5-fold in oocytes injected with 20 ng of a size-fractionated human renal cortex mRNA fragment (2–3 kb) in comparison with water-injected oocytes. Na$^+$-dependent thymidine uptake in oocytes injected with the 2–3 kb mRNA fragment was inhibited significantly by thymidine and guanosine but not by formycin B, consistent with the N4 Na$^+$-nucleoside cotransporter. The K_m (28 μM) of Na$^+$-dependent thymidine uptake in the oocytes injected with the 2–3 kb mRNA fragment was similar to the K_m (27 μM) of Na$^+$-dependent thymidine uptake obtained in human renal brush border membrane vesicles. These data suggest for the first time that a Na$^+$-nucleoside cotransporter from human kidney can be expressed in *X. laevis* oocytes.

Key words: sodium nucleoside transporter; human kidney; *Xenopus laevis* oocytes; expression system; biological transport; renal brush border membrane

Nucleosides are transported into cells via equilibrative and secondary active Na$^+$-dependent transport systems [1]. Four subtypes of Na$^+$-dependent nucleoside transport systems have been characterized in mammalian cells. N1 (or cif), a purine-selective transporter, and N2 (or cit), a pyrimidine-selective transporter, have been characterized in bovine kidney and in a mouse intestinal epithelial cell line [2, 3]. N3, a broadly selective transporter, has been characterized in rabbit choroid plexus [4] and more recently in Caco-2 cells [5]. Recently, we characterized a Na$^+$-nucleoside transport system, tentatively designated N4, in the brush border membrane of the human renal cortex [6, 7]. N4 is selective for pyrimidine nucleosides but also transports adenosine and guanosine. Unlike N3, N4 excludes inosine and formycin B.

The overall goal of this study was to demonstrate functional expression of Na$^+$-nucleoside transport activity in *Xenopus laevis* oocytes injected with mRNA (poly(A)$^+$ RNA) obtained from human kidney. An enhanced uptake was observed in oocytes injected with mRNA from human kidney versus those injected with water. The uptake was maximally enhanced in oocytes injected with a 2–3 kb fragment of mRNA. The substrate selectivity and kinetics of the expressed transporter are consistent with that of the N4, Na$^+$-nucleoside cotransporter.

Materials and Methods

Isolation and size-fractionation of poly(A)$^+$ RNA. Human kidneys were obtained from the Organ/Tissue Transplant Services at the University of California San Francisco. Total RNA was isolated from the outer cortex by the Trizol™ extraction method in accordance with the protocol given by Gibco BRL. Poly(A)$^+$ RNA (total mRNA) was isolated by oligo(dT)-cellulose (Boehringer Mannheim) chromatography according to the manufacturer's protocol and precipitated with 70% ethanol. Total mRNA was size-fractionated using a 6–20% sucrose gradient in EDTA (1 mM) and Tris (10 mM). The individual mRNA fractions were dissolved in water (0.4–1 μg/μL) and stored at −70° until used.

Gel electrophoresis. The size range of mRNA in each fraction was determined by electrophoresis on a 0.9% (w/v) agarose gel containing 0.37 M formaldehyde. RNA fractions were labeled with ethidium bromide (0.1 mg/mL) and resolved by electrophoresis at room temperature at a constant voltage of 5 V/cm for 2 hr. The sizes of mRNAs in different fractions were calculated by reference to BRL RNA molecular weight standards (Life Technologies, Inc., Burlington, Ontario, Canada).

Oocytes and microinjection. All methods and techniques regarding the handling of oocytes have been described previously [8–10]. Oocytes (stages 5 and 6) were isolated and maintained in modified Barth's medium overnight at 18°. Healthy oocytes were injected with 50 nL of total mRNA (1 ng/nL) or size-fractionated mRNA (0.4 ng/nL) using a semiautomatic injector. Control oocytes were injected with 50 nL of water. The injected oocytes were kept in Barth's medium (18°) for 4 days prior to uptake experiments.

Thymidine uptake. The uptake of [^{3}H]thymidine (65 Ci/mmol, Moravek Biochemicals Inc., Brea, CA) in mRNA-injected oocytes was studied using previously described methods [8,10]. Briefly, the oocytes were incubated for 30 min with 200 μL of reaction mixture containing [^{3}H]thymidine (50 μCi/mL) and unlabeled thymidine (10 μM). For Na$^+$-dependent studies, the reaction mixture contained NaCl (100 mM) and for Na$^+$-independent studies, the mixture contained choline chloride (100 mM). For inhibition studies, various unlabeled nucleosides (100 μM) were added to the reaction mixture. After incubation, the oocytes were washed with

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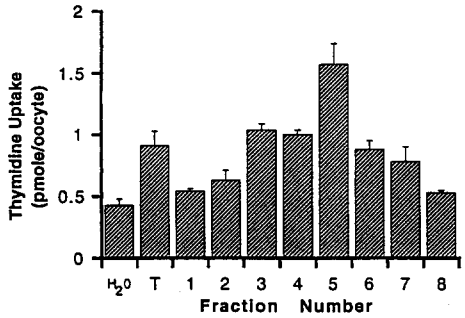

Fig. 1. Na^+-dependent uptake of thymidine in oocytes injected with size-fractioned mRNA. Thymidine uptake (30 min) was determined in *X. laevis* oocytes injected with 50 nL of water (H_2O), 50 nL of a solution of poly(A)$^+$ RNA (1 ng/nL in water) (T), or 20 ng of size-fractionated mRNA (fractions 1–8) prepared from human renal cortex. The size of mRNA ranged from 0.8–2.1 kb (fraction 8) to 3.8–5.0 kb (fraction 1). Each bar represents the mean ± SD of data obtained in two experiments of 5–9 oocytes per experiment. Na^+-stimulated thymidine uptake in fractions 2–7 was significantly different from its uptake in water-injected oocytes ($P < 0.05$).

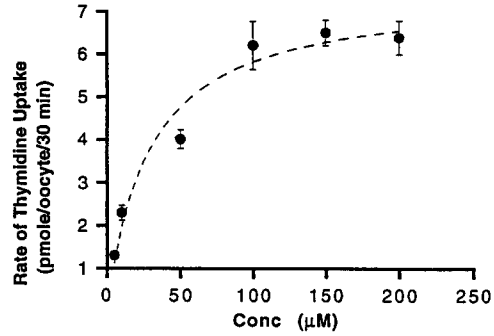

Fig. 2. Concentration dependence of Na^+-dependent thymidine uptake in mRNA-injected oocytes. The rate of thymidine uptake in the presence of Na^+ was determined 4 days after oocytes were injected with a size fraction mRNA species (2–3 kb). The curve represents the computer-generated fit to the equation: $[v = (V_{max}.C)/(K_m \pm C)]$. Each point is expressed as a mean ± SD of uptake values in oocytes (10–15) from two separate experiments.

3 × 3.5 mL Na^+-free medium. Each oocyte was then dissolved in 10% SDS, and radioactivity was assayed by liquid scintillation counting.

Data analysis. For each experiment, uptake values were obtained in 5–9 oocytes and expressed as pmol/oocyte/30 min. In general, two experiments were performed, and uptake is expressed as a mean ± SD of data obtained from the combined experiments (10–20 oocytes). Statistical analysis was carried out by an unpaired Student's *t*-test, where $P < 0.05$ was considered significant.

Results

In oocytes injected with mRNA isolated from human kidney, a significant enhancement ($P < 0.05$) of thymidine uptake (2–3-fold) in the presence of Na^+ was observed as compared with water-injected oocytes (Fig. 1). In contrast, the Na^+-independent uptake of thymidine was not enhanced significantly in the mRNA-injected oocytes (data not shown). The Na^+-dependent uptake of [^{3}H]thymidine in oocytes injected with 20 ng of size-fractionated mRNA species (fractions 2–7) was significantly higher ($P < 0.01$) than in water-injected oocytes. The maximum enhancement (4–5-fold) was observed in fraction 5. This corresponds to a size fraction containing mostly 2–3 kb mRNA species. In contrast, Na^+-independent uptake of thymidine was not enhanced in oocytes injected with the individual fractions as compared with water-injected oocytes (data not shown). Subsequent experiments were performed in oocytes injected with fraction 5.

The kinetics of Na^+-thymidine uptake were determined in oocytes injected with 20 ng of fraction 5. A representative experiment is shown in Fig. 2. Since uptake of thymidine was linear for the first hour (data not shown), 30 min was used to determine the initial rate of transport. In the presence of Na^+, the transport process was saturable. The K_m and V_{max} obtained were 28.2 ± 4.2 μM and 7.5 ± 0.8 pmol/oocyte/30 min, respectively. Na^+-independent uptake of thymidine was not significantly different from uptake values obtained in water-injected oocytes.

The uptake of thymidine in the presence of Na^+ was inhibited significantly ($P < 0.05$) by thymidine (100 μM) and guanosine (100 μM) but not by formycin B (100 μM)

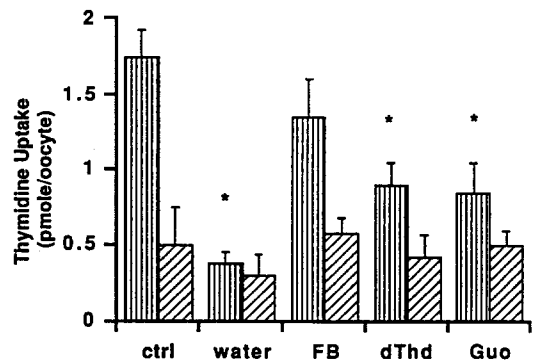

Fig. 3. Inhibition of Na^+-dependent thymidine uptake in mRNA-injected *X. laevis* oocytes. The uptake of thymidine (10 μM) was determined at 30 min in oocytes injected with size-fraction 5 alone (ctrl) and in the presence of 100 μM formycin B (FB), thymidine (dThd), or guanosine (Guo). The bars represent data obtained in the presence (vertical lines) or absence (slated lines) of Na^+. Also shown are data obtained in water-injected oocytes. Each bar represents the mean ± SD of uptake values in oocytes (10–15) from two separate experiments. Key: (*) $P < 0.05$ vs control.

in oocytes injected with fraction 5 obtained from human renal cortex (Fig. 3). In contrast, Na^+-independent thymidine uptake was not inhibited significantly by any of these compounds.

Discussion

The overall goal of this study was to develop an expression system in *X. laevis* oocytes for the N4 Na^+-nucleoside transport system in human renal cortex as an initial step in the cloning process of this protein. Previously, oocytes have been used to express the activity of N1, N2 and N3 Na^+-nucleoside transport systems from rat jejunum

[11], rat renal cortex [8], and rabbit choroid plexus [10]. Injection of total mRNA (50 ng) from human renal cortex into oocytes resulted in a 2–3-fold enhancement of [^{3}H]thymidine uptake in the presence of Na^+ (Fig. 1), which suggests that mRNA contained a message that encodes for a Na^+-nucleoside transport system.

Previously, Huang *et al.* [11] size-fractionated mRNA from rat jejunum and found that N1 and N3 Na^+-nucleoside transport activities were maximally expressed in oocytes injected with an mRNA fragment with an average size of 2.3 kb. In addition, a cDNA (2.3 kb) encoding a putative Na^+-nucleoside transporter was isolated from a rabbit renal cDNA library [12]. In our studies, Na^+-dependent thymidine uptake was maximally expressed in oocytes injected with fraction 5 (2–3 kb). The size of the mRNA species is consistent with that of other studies [11, 12]. Previously, Na^+–thymidine uptake was reported to be enhanced in oocytes injected with a 2–3 kb size-fractionated mRNA species from human renal cortex [8]. However, the characteristics of the expressed uptake were not examined, and the subtype (N1, N2, N3 and N4) of Na^+-nucleoside transport was not determined. A Na^+-nucleoside cotransporter (N2) from rabbit intestine has also been expressed in *X. laevis* oocytes [13].

The rate of thymidine uptake in oocytes injected with fraction 5 as a function of increasing thymidine concentration was saturable with a K_m of 28.2 ± 4.2 μM and a V_{max} of 7.5 ± 0.8 pmol/mg protein/30 min. This K_m correlates well with the K_m of Na^+–thymidine uptake (27.4 ± 10.8 μM) in human renal brush border membrane vesicles [7]. It is important to note that the Na^+-independent component and the endogenous Na^+-dependent component of thymidine uptake in the oocytes were not subtracted in the kinetic analysis due to the difficulty in resolving these components as a result of a low level of enhancement of Na^+-dependent thymidine uptake in mRNA-injected oocytes. Thymidine and guanosine but not formycin B significantly inhibited Na^+-dependent thymidine uptake in oocytes injected with fraction 5. These data are consistent with the substrate selectivity of N4. This is the first study demonstrating the functional expression of a human renal Na^+-nucleoside cotransporter in *X. laevis* oocytes.

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REFERENCES

1. Plagemann PGW, Wohlhueter RM and Woffendin C, Nucleoside and nucleobase transport in animal cells. *Biochim Biophys Acta* **947**: 405–443, 1988.
2. Williams TC and Jarvis SM, Multiple sodium-dependent nucleoside transport systems in bovine renal brush border membrane vesicles. *Biochem J* **274**: 27–33, 1991.
3. Vijayalakshmi D and Belt JA, Sodium-dependent nucleoside transport in mouse intestinal epithelial cells: Two transport systems with differing substrate specificities. *J Biol Chem* **263**: 19419–19423, 1988.
4. Wu X, Yuan G, Brett CM, Hui AC and Giacomini KM, Sodium-dependent nucleoside transport in choroid plexus from rabbit: Evidence for a single transporter for purine and pyrimidine nucleosides. *J Biol Chem* **267**: 8813–8818, 1992.
5. Belt JA, Marina NM, Phelps DA and Crawford CR, Nucleoside transport in normal and neoplastic cells. *Adv Enzyme Regul* **33**: 235–252, 1993.
6. Gutierrez MM, Brett CM, Ott RJ, Hui AC and Giacomini KM, Nucleoside transport in brush border membrane vesicles from human kidney. *Biochim Biophys Acta* **1105**: 1–9, 1992.
7. Gutierrez MM and Giacomini KM, Substrate selectivity, potential sensitivity and stoichiometry of Na^+-nucleoside transport in brush border membrane vesicles from human kidney. *Biochim Biophys Acta* **1149**: 202–208, 1993.
8. Giacomini KM, Markovich D, Werner A, Biber J, Wu X and Mürer H, Expression of a renal Na^+-nucleoside cotransporter system (N2) in *Xenopus laevis* oocytes. *Pflugers Arch* **427**: 381–383, 1994.
9. Werner A, Biber J, Forgo J, Palacin M and Mürer H, Expression of renal transport systems for inorganic phosphate and sulfate in *Xenopus laevis* oocytes. *J Biol Chem* **265**: 12331–12336, 1990.
10. Wu X and Giacomini KM, Expression of the choroid plexus sodium-nucleoside cotransporter (N3) in *Xenopus laevis* oocytes. *Biochem Pharmacol* **48**: 432–434, 1994.
11. Huang Q-Q, Harvey CM, Paterson ARP, Cass CE and Young JD, Functional expression of Na^+-dependent nucleoside transport systems of rat intestine in isolated oocytes of *Xenopus laevis*. *J Biol Chem* **268**: 20613–20619, 1993.
12. Pajor AM and Wright EM, Cloning and functional expression of a mammalian Na^+/nucleoside cotransporter. *J Biol Chem* **267**: 3557–3560, 1992.
13. Jarvis SM and Griffith DA, Expression of the rabbit intestinal N2 Na^+/nucleoside transporter in *Xenopus laevis* oocytes. *Biochem J* **278**: 605–607, 1991.