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Expression of the choroid plexus sodium-nucleoside cotransporter (N3) in *Xenopus laevis* oocytes

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Abstract—In this study, we determined the expression of the Na^+-nucleoside cotransporter (N3) in *Xenopus laevis* oocytes injected with poly(A)$^+$ RNA isolated from rabbit choroid plexus. The Na^+-dependent thymidine uptake in poly(A)$^+$ RNA-injected oocytes (maximum 4–5 days after injection) increased proportionally with the injected dose of poly(A)$^+$ RNA. Uptake was enhanced 4- to 5-fold in oocytes injected with 40 ng poly (A)$^+$ RNA in comparison to water-injected oocytes. Consistent with the N3 Na^+-nucleoside cotransporter, Na^+-dependent thymidine uptake in poly(A)$^+$ RNA-injected oocytes was inhibited significantly by both purine and pyrimidine nucleosides, but not by dideoxycytidine, a nucleoside analog modified on the ribose ring. These data suggest for the first time that the N3 Na^+-nucleoside cotransporter in rabbit choroid plexus can be expressed in *X. laevis* oocytes.

Key words: sodium nucleoside transporter (N3); rabbit choroid plexus; rat kidney; expression system; *Xenopus laevis* oocytes

Several subtypes of secondary active Na^+-nucleoside cotransport systems have been identified in a variety of cell types [1–7]. N3, first identified in rabbit choroid plexus and more recently in Caco 2 cells, is broadly selective for both purine and pyrimidine nucleosides [4, 7]. In the human renal brush border membrane, another system (termed N4) selective for pyrimidine nucleosides and guanosine has been characterized recently [5, 6].

The strategy of expression cloning in *Xenopus laevis* oocytes has been used successfully to clone several Na^+-dependent cotransporters [8, 9], and functional expression in *X. laevis* oocytes for intestinal and renal (N2) Na^+-nucleoside cotransport systems has been described [10–12]. The present study demonstrates that the N3 Na^+-nucleoside cotransporter can be functionally expressed in *X. laevis* oocytes injected with poly(A)$^+$ RNA isolated from rabbit choroid plexus.

Materials and Methods

Isolation of poly(A)$^+$ RNA. The choroid plexus tissue in the lateral ventricles was harvested from New Zealand White rabbits (Nitabell Rabbitry, Hayward, CA). Poly(A)$^+$ RNA was isolated from the choroid plexus by using a Micro-FastTrack™ mRNA isolation kit (In Vitrogen Co., San Diego, CA) and following the protocol provided by the manufacturer. Typically, 4–6 μg of poly(A)$^+$ RNA was isolated from eight rabbit brains and was stored in water at −70° until used.

Oocytes and microinjection. Oocytes were harvested from *X. laevis* (Xenopus, Ann Arbor, MI) and were defolliculated with collagenase at room temperature as previously described [11, 13]. Fully grown stages 5 and 6 oocytes were isolated and kept in modified Barth's solution overnight at 18°. Healthy looking oocytes were injected with 50 nL of either water or poly(A)$^+$ RNA (0.2 to 1 ng/nL in water) using a semiautomatic injector. The injected oocytes were maintained in the modified Barth's solution at 18° for up to 5 days with a daily change of Barth's solution.

Thymidine uptake. The uptake of [^{3}H]thymidine (65 Ci/mmol, Moravek Biochemicals, Inc., Brea, CA) in oocytes was measured by a method described previously [11, 13]. Briefly, the injected oocytes [4–9] were incubated with 200 μL of reaction mixture. Typically, the reaction mixture consisted of [^{3}H]thymidine (50 μCi/mL) and unlabeled thymidine (50 μM) in Na^+-containing buffer (100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES/Tris, pH 7.4) for Na^+-dependent uptake studies or in Na^+-free buffer (100 mM choline chloride replaced 100 mM NaCl) for Na^+-independent uptake studies. For inhibition studies, a 1 mM concentration of unlabeled nucleoside or ddC*, a nucleoside analog, was also included in the reaction mixture. In all the experiments, oocytes were incubated in reaction mixture for 30 min at 25°. Previous studies suggested that Na^+-thymidine uptake is linear for 1 hr [11]. After the incubation, each oocyte was placed separately into a scintillation vial, and 10% SDS was added to digest the oocyte. Scintillant was added to each vial, and the radioactivity was determined by liquid scintillation counting.

Data analysis. The uptake value is generally expressed as picomoles per oocyte·30 min and presented as a mean ± SEM obtained from 4 to 9 oocytes in each experiment or from separate experiments. Statistical analysis was carried out by unpaired Student's *t*-test, and a probability, P, of less than 0.05 was considered significant.

Results

In water-injected oocytes, thymidine uptake in the presence of Na^+ was significantly ($P < 0.05$) greater than that in the absence of Na^+ (data not shown), demonstrating the presence of an intrinsic Na^+-nucleoside cotransporter. NBMPR, an equilibrative nucleoside transport inhibitor, significantly ($P < 0.05$) inhibited both Na^+-dependent and Na^+-independent thymidine uptake in the uninjected oocytes, suggesting that the oocytes also have intrinsic Na^+-independent transporter.

Injection of poly(A)$^+$ RNA (40 ng) isolated from rabbit choroid plexus into oocytes resulted in a significant enhancement (4- to 5-fold) of Na^+-dependent but not Na^+-independent thymidine uptake (Fig. 1). Na^+-dependent uptake of thymidine increased with the incubation time after injection of poly(A)$^+$ RNA (50 ng) and was maximum on days 4–5. Na^+-dependent thymidine uptake was 2.04 ± 0.14 pmol/oocyte/30 min 5 days after injection of

* Abbreviations: ddC, dideoxycytidine; NBMPR, nitrobenzylthioinosine; *cif*, concentrative nucleoside transporter that is insensitive to 6-thiopurines and uses formycin B as a substrate; and *cit*, concentrative nucleoside transporter that is insensitive to 6-thiopurines and that uses thymidine as a substrate.

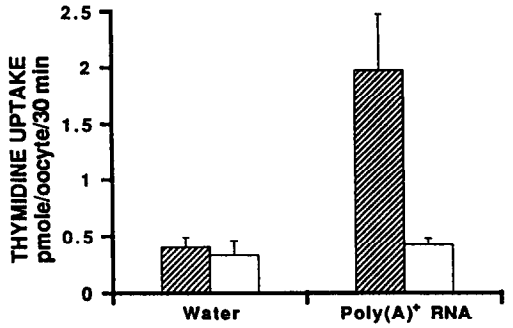

Fig. 1. Expression of the Na^+-nucleoside cotransporter in *X. laevis* oocytes. Oocytes were injected with 50 nL of water (i.e. vehicle) or a solution containing 40 ng of poly(A)$^+$ RNA from rabbit choroid plexus. The uptake of thymidine (50 μM) at 25° was determined 4 days after the injection in the presence (hatched bars) or absence (open bars) of Na^+. Data represent the means ± SEM from four experiments with different batches of oocytes and poly(A)$^+$ RNA.

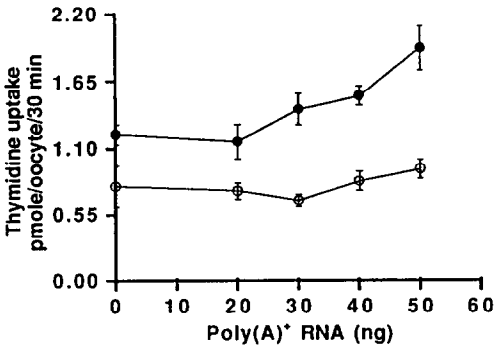

Fig. 2. Dose-dependence of thymidine uptake in *X. laevis* oocytes. Oocytes were injected with 50 nL of solutions that contained 0–1 ng of poly(A)$^+$ RNA from rabbit choroid plexus/nL of water. The uptake of thymidine (50 μM) at 25° was measured 4 days after the injection in the presence (●) or absence of Na^+ (○). Data represent the means ± SEM from 6 to 9 oocytes.

poly(A)$^+$ RNA, and was significantly greater than the value of 1.30 ± 0.10 pmol/oocyte/30 min obtained 2 days after injection ($P < 0.05$). In contrast, Na^+-independent uptake of thymidine was unaffected by the incubation time (data not shown).

Data shown in Fig. 2 demonstrate that Na^+-dependent uptake of thymidine in oocytes was dependent upon the dose of poly(A)$^+$ RNA injected. In oocytes injected with 40 and 50 ng of poly(A)$^+$ RNA, Na^+-dependent thymidine uptake was significantly greater than the value for water-injected oocytes ($P < 0.01$). Na^+-independent uptake of thymidine did not increase significantly with the dose of poly(A)$^+$ RNA.

Consistent with an N3, Na^+-nucleoside cotransporter, both purine and pyrimidine nucleosides (1 mM), formycin B, guanosine, cytidine and thymidine, significantly inhibited Na^+-dependent thymidine uptake ($P < 0.01$), whereas ddC did not. Na^+-dependent thymidine uptake in water-injected oocytes was unaffected by any of these inhibitors (data not shown). Furthermore, none of the inhibitors used in this study significantly inhibited Na^+-independent uptake of thymidine in either water or poly(A)$^+$ RNA-injected oocytes (data not shown). Injection of poly(A)$^+$ RNA isolated from rat renal cortex resulted in the expression of Na^+-dependent thymidine uptake, which was inhibited significantly by the pyrimidine nucleosides (1 mM), cytidine and thymidine, but not by the purine nucleosides, guanosine and formycin B, or by ddC (Table 1).

Discussion

The goal of this study was to develop an expression system in *X. laevis* oocytes for the N3 Na^+-nucleoside cotransporter in rabbit choroid plexus as a first step toward its eventual cloning. Injection of poly(A)$^+$ RNA from rabbit choroid plexus into *X. laevis* oocytes resulted in a 5-fold enhanced Na^+-dependent uptake of thymidine over

Table 1. Inhibition of thymidine uptake in *X. laevis* oocytes by nucleosides

	Thymidine uptake (pmol/oocyte/30 min)	
	Choroid plexus poly(A)$^+$ RNA	Renal cortex poly(A)$^+$ RNA
Water injected		
Control	0.46 ± 0.10*	0.69 ± 0.05*
Poly(A)$^+$ RNA injected		
Control	2.13 ± 0.67	1.14 ± 0.11
Thymidine	0.37 ± 0.06*	0.76 ± 0.10*
Cytidine	0.51 ± 0.18*	0.54 ± 0.14*
Guanosine	0.38 ± 0.03*	1.06 ± 0.19
Formycin B	0.61 ± 0.33*	0.94 ± 0.05
Dideoxycytidine†	2.07 ± 0.27	1.20 ± 0.3

Oocytes were injected with 40 ng (in 50 nL of water) of poly(A)$^+$ RNA from rabbit choroid plexus or from rat renal cortex. The Na^+-dependent uptake of thymidine (50 μM) was determined 4 days after the injection in the presence and absence of nucleosides and nucleoside analogs (1 mM). Data represent the means ± SEM from 3 to 8 oocytes in each of two to three separate experiments unless noted otherwise.

* Statistically different from the poly(A)$^+$ RNA-injected control for the given source, $P < 0.05$.

† Values for dideoxycytidine represent data from 3 to 8 oocytes in one experiment.

background (Fig. 1), suggesting that the injected poly(A)$^+$ RNA contains a message that encodes a Na^+-nucleoside membrane transport protein.

The Na^+-nucleoside transporter in the rabbit choroid plexus, termed N3, differs from previously described purine and pyrimidine selective transporters termed N1 or *cif* and N2 or *cit*, respectively. A recent study suggests that N3 is also present in a human colon carcinoma cell line (Caco 2) [7]. Apparent N3 activity has been expressed recently in *X. laevis* oocytes injected with mRNA from rat jejunem [12]; however, it is not known whether N3 plays a functional role in the jejunem. The data obtained in this study demonstrating that both purine and pyrimidine nucleosides significantly inhibit Na^+-thymidine uptake in poly(A)$^+$ RNA-injected oocytes suggest that the expressed activity represents the N3 Na^+-nucleoside cotransporter. Direct comparison of the inhibition pattern of Na^+-thymidine uptake (Table 1) suggests that an N2 transporter is expressed in oocytes injected with poly(A)$^+$ RNA from rat kidney and an N3 transporter is expressed in oocytes injected with poly(A)$^+$ RNA from rabbit choroid plexus. Furthermore, the expressed activity differs from the previously reported N2 activity [10–12] in that we could not demonstrate a reduction of Na^+-thymidine uptake by the addition of ddC in oocytes injected with poly(A)$^+$ RNA from rabbit choroid plexus. This observation suggests that the expressed N3 transporter is not the same as the transporter expressed following injection of a chimeric cRNA purportedly encoding a Na^+-nucleoside cotransporter [14].

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Schools of Pharmacy and Medicine
University of California
San Francisco, CA 94143, U.S.A.

XIAOCHUN WU
KATHLEEN M. GIACOMINI*

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* Corresponding author: Kathleen M. Giacomini, Ph.D. Department of Pharmacy, University of California, 513 Parnassus Ave., S-926, Box 0446, San Francisco, CA 94143-0446. Tel. (415) 476-1936; FAX (415) 476-0688.