Functional expression of the nitrobenzylthioinosine-sensitive nucleoside transporter of human choriocarcinoma (BeWo) cells in isolated oocytes of *Xenopus laevis*

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Cultured human choriocarcinoma (BeWo) cells have previously been shown to exhibit, in comparison with other cultured cell types, elevated nitrobenzylthioinosine (NBMPR)-sensitive transport activity and large numbers ($> 10^7$ /cell) of high-affinity NBMPR-binding sites [Boumah, Hogue and Cass (1992) *Biochem. J.* **288**, 987–996]. The present study investigates whether NBMPR-sensitive nucleoside transport activity could be induced in *Xenopus laevis* oocytes by microinjection of poly(A)⁺ RNA isolated from proliferating cultures of BeWo cells. Expression of uridine transport activity was assayed by comparing rates of uptake (22 °C) of 100 μ M [³H]uridine by RNA-injected oocytes with uptake by water-injected or uninjected oocytes. A 4-fold stimulation of uridine uptake (2.0 versus 0.5 pmol/90 min per oocyte) was seen when oocytes were injected with 50 ng of BeWo poly(A)⁺ RNA, and this stimulation was abolished when the RNA-injected oocytes were assayed in the presence of 10 μ M

NBMPR. The expressed uridine transport activity in oocytes was highly sensitive to NBMPR, with a 50% reduction seen at 1.1 nM NBMPR (IC₅₀ value). The IC₅₀ value for NBMPR inhibition of uptake of 100 μ M [³H]uridine by intact BeWo cells was 1.4 nM. Inward fluxes of [³H]uridine in the RNA-injected oocytes were greatly reduced in the presence of high concentrations (2 mM) of non-radioactive nucleosides (adenosine, thymidine, inosine) that are known permeants of NBMPR-sensitive nucleoside transport processes. These results establish that the abundance of NBMPR-sensitive nucleoside transporter mRNA in poly(A)⁺ RNA preparations from BeWo cells is sufficient to achieve production of functionally active transporter protein in *Xenopus* oocytes and that, when expressed in *Xenopus* oocytes, the transporters exhibit NBMPR sensitivity and permeant selectivity similar to that of the native transporters.

INTRODUCTION

Nucleosides enter animal cells by carrier-mediated processes that are either equilibrative or concentrative in nature. The two equilibrative nucleoside transport (NT) processes that have been identified exhibit similar permeant selectivities but differ in their kinetic properties and sensitivities to inhibition by low concentrations of nitrobenzylthioinosine (NBMPR). The equilibrative NBMPR-sensitive transporters, termed *es*, are inhibited by NBMPR concentrations in the 1 nM range, whereas the equilibrative NBMPR-insensitive transporters, termed *ei*, are unaffected by 1 μ M NBMPR [1–3]. The three distinct sodium-dependent NT processes that have been identified [4–8] are insensitive to inhibition by NBMPR and are recognized by their permeant selectivities, transporting primarily purine nucleosides (*cif* or N1), primarily pyrimidine nucleosides (*cit* or N2) or a broad range of both purine and pyrimidine nucleosides (*cib* or N3). Except for human erythrocytes and a few cultured cell lines, most cell types that have been studied exhibit more than one NT process, making elucidation of molecular properties of the individual transporters difficult.

The *es* transporter of human erythrocytes has been identified as a heterogeneously glycosylated (45–65 kDa) membrane glycoprotein that co-migrates with the equilibrative glucose transporter (GLUT-1) during SDS/PAGE [9]. It has been purified to near-homogeneity [10,11] and used to produce polyclonal anti-

bodies that also recognize the *es* transporters of pig and rabbit erythrocytes [12]. Studies with human erythrocytes have shown that NBMPR inhibits *es* NT activity by binding tightly (K_d 1 nM) and specifically to the 55 kDa protein [9,13,14]. NBMPR-binding-site abundance is frequently taken as a measure of functional *es* transporters, since the total number of NBMPR-binding sites is proportional to V_{max} values for uridine influx in erythrocytes of several mammalian species [15].

Cultured human choriocarcinoma (BeWo) cells have been studied mainly for their ability to undergo differentiation from proliferating cytotrophoblast-like cells to larger syncytiotrophoblast-like cells when exposed to methotrexate, a process that resembles normal *in utero* development [16,17]. Recently we found that BeWo cells possess extraordinarily large numbers of NBMPR-binding sites, ranging from 10^7 to 3×10^7 sites per cell (approx. 5 pmol/mg of protein), depending on culture conditions [18]. Comparison of thymidine transport kinetics with those of other cultured cell types indicated that BeWo cells also exhibit unusually high levels of NBMPR-sensitive thymidine transport activity [18]. These characteristics raised the possibility that BeWo cells might express higher levels of *es* transporter mRNA than other cell types and thus be a candidate cell type from which to clone cDNA sequences encoding *es* transporter proteins.

Xenopus laevis oocytes are widely used for heterologous expression of proteins since, upon injection into oocytes, foreign mRNAs are rapidly translated into proteins that appear to be

Abbreviations used: *es*, equilibrative-sensitive; *ei*, equilibrative-insensitive; *cib*, concentrative-insensitive-broad; *cif*, concentrative-insensitive-formycin B; *cit*, concentrative-insensitive-thymidine; MBM, modified Barth's medium; NBMPR, nitrobenzylthioinosine (6-[(4-nitrobenzyl)thio]-9- β -D-ribofuranosylpurine); NT, nucleoside transport.

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correctly processed and frequently exhibit functional properties that are similar to those of the native proteins. Functional expression in *Xenopus* oocytes has been successfully used for studies of cDNAs encoding membrane proteins [19–21] and for isolation of cDNAs encoding rare membrane proteins for which the more conventional approaches would be very difficult (for review, see [22]).

In the current work, poly (A)⁺ RNA was isolated from actively proliferating cultures of BeWo cells that were grown under conditions shown previously [18] to favour expression of high levels of NBMPR-binding activity. Mature (Stage VI) oocytes from *Xenopus laevis* were microinjected with BeWo RNA or with water and, after 5 days, were assayed for expression of uridine transport activity by measuring the uptake of 100 μ M [³H]uridine by individual oocytes. The 'expressed' activity was characterized by examining its sensitivity to inhibition by NBMPR and nucleosides that are known to be permeants of *es*-mediated NT processes. Parallel studies were conducted in adherent cultures of BeWo cells, since uridine transport has not previously been studied in these cells. Our results, which demonstrated that the NBMPR sensitivity and permeant selectivity of the expressed BeWo transporters were similar to those of the native transporters, establish a strategy for molecular cloning of human *es* transporter cDNA. Expression of Na⁺-dependent NT activities in *Xenopus* oocytes after microinjection of mRNA preparations from intestinal epithelia of rabbit [23] and rat [24], and by cRNA encoded by a rabbit kidney cDNA [25], has been reported recently.

EXPERIMENTAL

Cell culture

The origin and characteristics of BeWo cells have been described [16,17]. Cultures were initiated from *Mycoplasma*-free frozen stocks and grown at 37 °C in a humidified atmosphere of 5 % CO₂ in air as described previously [17]. Cells were harvested at weekly intervals from proliferating cultures by trypsin treatment and maintained in plastic culture flasks in Roswell Park Memorial Institute (RPMI) 1640 basal medium supplemented with 5 % fetal bovine serum and 5 % Nu-serum type IV. Cell numbers were determined with an electronic particle counter.

For transport experiments, replicate cultures were established in plastic culture dishes (60 mm × 15 mm) at 0.5×10^5 to 1.0×10^5 cells per dish and used 3–4 days later. For isolation of mRNA, cultures were established in roller bottles at 5×10^6 cells per bottle and used 3–4 days later.

Transport assay

Uridine fluxes were determined at room temperature (22–23 °C) by a procedure previously used in studies of thymidine transport by BeWo cells [18]. Uptake assays were (i) initiated by addition of 1.5 ml of [³H]uridine in BeWo transport buffer (20 mM Tris/HCl, 3 mM K₂HPO₄, 1 mM MgCl₂·6H₂O, 1.8 mM CaCl₂, 5 mM glucose, 130 mM NaCl, pH 7.4), and (ii) stopped by aspiration of the medium and immersion of the dish in a large volume (1 litre) of ice-cold PBS (137 mM NaCl, 5.36 mM KCl, 1.1 mM KH₂PO₄ and 1.1 mM Na₂HPO₄, pH 7.2) that contained 100 μ M of the nucleoside transport inhibitor dilazep. Uptake at time zero was determined with cells that had been incubated at 4 °C for 10 min with BeWo transport medium that contained 100 μ M dilazep. Each culture was processed individually and uptake intervals were timed by metronome signals. The radio-

active content of cultures (three per condition) was determined as described [18].

The effects of NBMPR on uridine fluxes were determined as described above using cultures that had been cooled to room temperature, rinsed twice with BeWo binding medium (RPMI 1640 without bicarbonate and supplemented with 9.6 mM NaCl, 5 mM glucose and 20 mM Hepes, pH 7.4) and incubated (10 ml per dish) for 2 h in BeWo binding medium alone or with NBMPR (0.1 nM to 20 μ M).

mRNA isolation

BeWo cells were harvested from roller bottles by trypsinization [0.05 % (w/v) trypsin, 0.02 % (w/v) EDTA, 37 °C, 20 min] and collected by centrifugation. Cell pellets were homogenized in 5 vol. of denaturing solution [4.0 M guanidinium thiocyanate, 0.1 M Tris/HCl, pH 7.5, 1 % (v/v) 2-mercaptoethanol, 0.5 % (w/v) sarcosyl]. Total RNA was prepared by centrifugation in caesium chloride gradients [26], and poly(A)⁺ mRNA was selected by two cycles of chromatography on oligo(dT)-cellulose (Type 7) according to the procedures recommended by the supplier (Pharmacia LKB Biotechnology, Baie D'Urfe, Quebec, Canada). The resulting poly(A)⁺ RNA was precipitated with ethanol, resuspended in diethyl pyrocarbonate-treated water at a concentration of 1.0 mg/ml, and stored at –70 °C.

Oocyte preparation and injection

Mature oocyte-containing *Xenopus laevis* (*Xenopus* I, Ann Arbor, MI, U.S.A.) were anaesthetized with ice and killed by a standard pithing procedure. The ovaries were removed, opened and washed into modified Barth's medium [MBM: 88 mM NaCl, 1 mM KCl, 0.33 mM Ca(NO₃)₂, 0.41 mM CaCl₂, 0.82 mM MgSO₄, 2.4 mM NaHCO₃, 2.5 mM sodium pyruvate, 5 mg/ml penicillin, 0.1 mg/ml gentamycin sulphate, 10 mM Hepes, pH 7.5]. Small clumps of ovarian tissue were dissected and incubated in 8 mg/ml collagenase (Type 1) at 20 °C for 1 h to separate oocytes from connective tissue and follicular layers. Mature, healthy stage VI oocytes were incubated at 18 °C in MBM for 24 h, when they were injected with either 50 nl of water containing BeWo mRNA (1 ng/nl) or 50 nl of water. The injected oocytes were incubated at 18 °C in MBM for 5 days and then assayed for expression of uridine transport activity.

Nucleoside uptake by oocytes

Uptake of 100 μ M [³H]uridine by injected oocytes was measured at 20 °C for 1 h (unless otherwise indicated) in *Xenopus* transport buffer (100 mM NaCl, 2 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM Hepes, pH 7.5). In some experiments the transport buffer also contained a non-radioactive nucleoside (adenosine, thymidine, uridine or inosine). Incubation intervals were ended by a succession of five rapid (< 3 min altogether) washes with ice-cold *Xenopus* transport buffer. In experiments that assessed the effects of NBMPR on uridine transport activity, oocytes were first incubated for 20 min at room temperature in *Xenopus* transport buffer alone or with graded concentrations of NBMPR, and then in *Xenopus* transport buffer that contained 100 μ M [³H]uridine alone or with the same graded concentrations of NBMPR. Individual oocytes were dissolved in 5 % (w/v) SDS for determination of cell-associated radioactivity by liquid scintillation counting.

Results are presented as means $\pm$ S.E.M. of determinations from nine or more individual oocytes. Where differences of means are given (i.e. net expressed fluxes), the standard error of

the difference between the means (S.E.D.M.) is presented. The significance of difference was tested using Student's *t* test and appropriate tables of probability.

Materials

[5,6-³H]Uridine (40 Ci/mmol; 1 Ci = 37 GBq) was obtained from Moravsek Biochemicals, Inc. (Brea, CA, U.S.A.) and was repurified to > 96% radiochemical purity by h.p.l.c. NBMPR and collagenase were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Dilazep was a gift from F. Hofmann-La Roche and Co. (Basel, Switzerland). Nu-serum type IV was obtained from Collaborative Research (Bedford, MA, U.S.A.) and other cell culture reagents were from Gibco (Burlington, Ont., Canada). Other reagents were purchased from standard commercial sources.

RESULTS AND DISCUSSION

Cells of the human choriocarcinoma line (BeWo) have the largest number of NBMPR-binding sites ever reported (10^7 – 3×10^7 sites/cell, depending on culture conditions) and also exhibit very high levels of thymidine transport activity, 90% of which is *es*-mediated [18]. Before attempting to express BeWo NT activity in *Xenopus* oocytes, we first examined the capacity of BeWo cells to transport uridine, which is a substrate for all of the known NT systems (Figure 1). It is evident from Figure 1(a), which shows representative time courses of uptake of [³H]uridine by BeWo cells incubated with and without 1 μ M NBMPR, that uridine uptake occurred primarily by an NBMPR-sensitive route. In the absence of NBMPR, cell-associated radioactivity increased rapidly with time, whereas in its presence radioactivity increased only slightly. Studies with other cell types have shown that 1 μ M NBMPR selectively inhibits the *es* transporter, leading to substantial reductions in cellular uptake of nucleoside permeants [3,27,28].

The concentration-dependence of NBMPR inhibition of uridine fluxes in BeWo cells was determined in the experiments shown in Figure 1(b). Cultures were first incubated with graded concentrations of NBMPR under conditions shown earlier [18] to be sufficient to allow an equilibrium to be reached between free NBMPR and the transport-inhibitory binding sites. Inward fluxes of 100 μ M [³H]uridine were then determined in the presence of exactly the same (i.e. equilibrium) concentrations of NBMPR. As was shown previously for thymidine [18], the primary route of influx was evidently via the *es* transporter, since fluxes were reduced to less than 10% of uninhibited (control) values by 10 nM NBMPR. The NBMPR concentration that reduced the NBMPR-sensitive component of uridine influx by 50% (*IC*₅₀ value) was 1.4 nM, very close to that observed previously (1.6 nM) for NBMPR inhibition of *es*-mediated thymidine transport by BeWo cells [18].

To determine if the BeWo *es* transporter could be expressed in *Xenopus* oocytes, poly(A)⁺ RNA was isolated from BeWo cells and injected into mature oocytes, and, since others have found that expression of membrane proteins in *Xenopus* oocytes reaches a peak 5–7 days after mRNA injection [29,30], the injected oocytes were cultured for 5 days before being assayed for transport activity. Uptake of [³H]uridine was measured in oocytes that had been injected with BeWo mRNA and, as controls, those that had been either not injected or injected with water. In the representative experiment presented in Figure 2, the mRNA-injected oocytes exhibited enhanced uptake of uridine, relative to

that of both types of controls, that was evident within 5 min and that reached 1.9–2.0 pmol/oocyte after 90 min. There was no difference in the results obtained with the uninjected and water-

Figure 1 Inhibition of uridine uptake into BeWo cells by NBMPR

(a) Replicate cultures (1.2×10^6 cells/dish) were first incubated with (●) or without (○) 1 μ M NBMPR and then assayed for uptake of 100 μ M [³H]uridine as described in the Experimental section. Each point represents the mean ($\pm$ S.D.) of three determinations; the absence of error bars indicates that S.D. values were less than the size of the symbols. Fluxes (pmol/s per 10^6 cells) in the absence (7.6) and presence (0.7) of NBMPR were estimated from uptake rates during the first 3 s of exposure to [³H]uridine. (b) Cultures (0.8×10^6 cells/dish) were first incubated for 2 h at 22 $^{\circ}$ C in 10 ml of BeWo binding medium that contained NBMPR at the concentrations indicated and then, after removing the binding medium by aspiration, assayed for uptake of 100 μ M [³H]uridine in BeWo transport media that contained the same NBMPR concentrations. Fluxes were estimated from uptake intervals between 0 and 3 s, and the values obtained in the presence of NBMPR are presented as percentage of the value (13.6 pmol/s per 10^6 cell) obtained in the absence of NBMPR (control).

Figure 2 Time course of uptake of uridine into oocytes of *Xenopus laevis* after injection of BeWo RNA

Oocytes were isolated as described in the Experimental section and were not injected (●), or were injected with 50 nl water alone (○) or with water that contained poly(A)⁺ RNA extracted from BeWo cells (▽). The oocytes were then incubated at 18 °C for 5 days in MBM. Uptake of 100 μM [³H]uridine was measured at 20 °C as described in the Experimental section. Values shown are means (± S.E.M., *n* = 10); the absence of error bars indicates that S.D. values were less than the size of the symbols. All oocytes came from one frog.

Table 1 Effect of NBMPR on expression of uridine transport activity by oocytes of *Xenopus laevis*

Oocytes (water-injected and mRNA-injected) were incubated for 20 min at 20 °C in *Xenopus* transport buffer in the absence or presence of 10 μM NBMPR, after which uptake of 100 μM [³H]uridine was measured at 20 °C for 1 h in the absence or presence of 10 μM NBMPR as described in the Experimental section. Values shown are means ± S.E.M. (*n* = 10). All oocytes came from one frog.

Oocytes	Uridine uptake (pmol/h per oocyte)	
	– NBMPR	+ NBMPR
Water-injected	0.38 ± 0.06	0.28 ± 0.04
mRNA-injected	1.80 ± 0.06	0.44 ± 0.02

injected oocytes; in both, uptake of uridine increased slightly with incubation time, reaching, after 90 min, a total accumulation of 0.4–0.5 pmol/oocyte. Thus, 5 days after injection of BeWo mRNA into *Xenopus* oocytes, there was a 4-fold difference in total uptake of uridine between the mRNA-injected and control oocytes, suggesting expression of BeWo nucleoside transporters in the mRNA-injected oocytes.

Although BeWo cells exhibited primarily *es* transport activity, a small component of NBMPR-insensitive (presumably *ei*) activity was also present (see Figure 1). The extent to which the enhanced uptake observed in the mRNA-injected oocytes was sensitive to NBMPR was examined in the experiment of Table 1, which compared the effects of 10 μM NBMPR on uridine fluxes in both mRNA- and water-injected oocytes during incubation in 100 μM uridine at 20 °C for 1 h. In mRNA-injected oocytes, treatment with NBMPR reduced uridine influx from 1.80 ± 0.06

Figure 3 Concentration-effect relationship for NBMPR inhibition of uridine uptake in oocytes of *Xenopus laevis* injected with BeWo mRNA

Oocytes were incubated in *Xenopus* transport buffer that contained NBMPR at the concentrations indicated, and then uptake of 100 μM [³H]uridine was measured in *Xenopus* transport buffer that contained the same NBMPR concentrations. Results are expressed as percentages of the flux (1.4 pmol/h per oocyte) observed in the absence of NBMPR (control).

to 0.44 ± 0.02 pmol/h per oocyte, a value that was slightly higher than the values observed in water-injected oocytes assayed in either the absence (0.38 ± 0.06 pmol/h per oocyte/h) or presence (0.28 ± 0.04 pmol/h per oocyte) of NBMPR. It is possible that the failure of NBMPR to reduce uridine fluxes in mRNA-injected oocytes to the level seen in water-injected oocytes was because the injection of BeWo mRNA also resulted in the expression of transport activity that was insensitive to NBMPR. In the experiment of Figure 1(b), approx. 5% of total uridine transport activity in intact BeWo cells was insensitive to inhibition by 20 μM NBMPR. The reduction in uptake observed after treatment of water-injected oocytes with NBMPR, although small, was statistically significant and suggested the presence of an endogenous NBMPR-sensitive component of uridine uptake in the oocytes.

A further indication that the uridine fluxes observed after injection of oocytes with BeWo mRNA were due to expression of the BeWo *es* transporter was provided by analysis of the concentration-dependence of NBMPR inhibition of fluxes in the mRNA-injected oocytes (Figure 3). As had been shown in the experiments with intact BeWo cells, the expressed uridine fluxes were reduced by ≥ 95% at NBMPR concentrations of 10–20 nM. In addition, the concentration that caused half-maximal inhibition (IC₅₀ value) of the expressed uridine fluxes in mRNA-injected oocytes was 1.1 nM, almost the same as the IC₅₀ value observed in the experiments with intact BeWo cells.

Having shown that the expressed uridine fluxes exhibited NBMPR sensitivity that was characteristic of the *es* transport process of BeWo cells, competition experiments with nucleosides known to be permeants of *es*-mediated transport were undertaken to determine if the expressed transporter exhibited the expected permeant selectivity. Both mRNA- and water-injected oocytes were incubated with 2 or 10 mM adenosine, uridine, thymidine or inosine just before and during the uptake assay with 100 μM [³H]uridine. Results from three such experiments (Table 2) show that net uridine fluxes were significantly reduced in the presence

Table 2 Effect of nucleosides on uptake of uridine into oocytes of *Xenopus laevis* injected with BeWo mRNA

Uptake of 100 μ M [3 H]uridine was measured at 20 °C for 1 h in oocytes (mRNA- and water-injected) in the absence or in the presence of 2 mM (Expt. 1) or of 10 mM (Expt. 2, Expt. 3) non-radioactive adenosine, inosine, thymidine and uridine. Values shown represent the differences (pmol/h per oocyte) between the fluxes observed in mRNA-injected oocytes and those observed in oocytes that had been injected with water. Values are means $\pm$ S.E.M. ($n = 20$). Numbers in parentheses represent 'percentage inhibition' of uptake obtained in the presence of the competitor relative to uptake in the absence of competitor (control). In each experiment, all oocytes came from one frog.

Competing nucleoside	Uridine uptake (pmol/h per oocyte)		
	Expt. 1	Expt. 2	Expt. 3
None	1.45 $\pm$ 0.10	0.69 $\pm$ 0.15	1.10 $\pm$ 0.36
Adenosine	0.18 $\pm$ 0.06 (87)	0.15 $\pm$ 0.10 (79)	0.35 $\pm$ 0.07 (69)
Inosine	0.40 $\pm$ 0.10 (71)	0.13 $\pm$ 0.07 (81)	0.21 $\pm$ 0.04 (81)
Thymidine	0.60 $\pm$ 0.10 (56)	0.09 $\pm$ 0.03 (87)	0.32 $\pm$ 0.09 (71)
Uridine	0.0 $\pm$ 0.07 (100)	0.31 $\pm$ 0.25 (55)	0.42 $\pm$ 0.12 (62)

of each of the competing nucleosides, a result that was consistent with expression of the *es* transporter, which accepts both purine and pyrimidine nucleosides as permeants. The results presented in Table 2 also demonstrate the experimental variability inherent in the oocyte expression studies. For example, net uridine fluxes obtained in the absence of competing nucleosides ranged from 0.69 to 1.45 pmol/h per oocyte, presumably reflecting biological differences in the oocytes themselves (each experiment was conducted with oocytes from a different frog) and/or in the abundance of *es*-like transporter mRNA in various poly(A)⁺ RNA preparations. (Throughout the course of this study, the stimulation of uridine uptake seen in the mRNA-injected oocytes, relative to water-injected oocytes, ranged from 1.5- to 4.5-fold, and in most experiments was about 2-fold). Furthermore, the extent of inhibition of mRNA-induced uptake of [3 H]uridine by various non-radioactive nucleosides (including uridine itself) varied from one experiment to the next, ranging from complete to partial inhibitions of 87–55%.

While it is possible that the expression induced in oocytes by injection of BeWo mRNA resulted from de-repression and/or activation of endogenous transport activity, the most likely explanation is that the uridine fluxes observed in mRNA-injected oocytes resulted from expression of the BeWo *es* transporter. In a separate study (C. E. Boumah, F. Y. P. Kwong, S. A. Baldwin, P. J. F. Henderson, J. D. Young, A. R. P. Paterson and C. E. Cass, unpublished work), we have isolated from a λ gt11 cDNA library of BeWo cells a short cDNA fragment that encodes an immunoreactive peptide recognized by polyclonal antibodies [12] raised against purified *es* transporter protein of human erythrocytes; co-injection of antisense RNA complementary to that cDNA probe completely blocked the expression of uridine transport activity in oocytes injected with BeWo mRNA, but not in oocytes that were injected with rat jejunal mRNA and that expressed only Na⁺-dependent uridine transport activities.

In conclusion, our results suggest that while oocytes possess endogenous uridine transport activity it is sufficiently low to permit detection of exogenous transport activity resulting from

expression of injected messages. Since the characteristics of the expressed uridine transport activity, namely NBMPR sensitivity with IC₅₀ values in the nanomolar range and inhibition by physiological nucleosides, were consistent with those of the *es* transporter of BeWo cells, we conclude that expression in *Xenopus* oocytes is a suitable approach to isolate and characterize cDNAs encoding the *es* transporter. The maximum levels of expressed *es* transport activity that could be achieved were relatively low, making detailed studies of functional characteristics (e.g. kinetics, permeant affinities) of the expressed transporter impractical. Such studies should be feasible with cloned *es* transporter cDNA.

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