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ORIGINAL ARTICLE

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Functional expression of the renal organic cation transporter and P-glycoprotein in *Xenopus laevis* oocytes

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Abstract The hypothesis that P-glycoprotein (P-gp) mediates the renal secretion of organic cations was tested by functional expression of mRNAs in the *Xenopus laevis* oocyte system. Efflux of 2'-deoxytubercidin (dTub), a substrate for the renal organic cation transporter (OCT) but not for P-gp, was enhanced by injection of renal mRNA but not by injection of mRNA from P-gp-overexpressing cells (MDCK cells transduced with the cDNA for human *MDR1*). The functional capacity of the MDCK-MDR mRNA was established by its ability to reduce the steady-state uptake of a classical P-gp substrate, vinblastine. Thus, these data indicate OCT and P-gp to be distinct entities. The *Xenopus* oocyte system provides a functional approach to further characterize the OCT.

Key words Oocytes · *Xenopus laevis* · Transport
P-glycoprotein · Renal · Deoxytubercidin
Vinblastine · mRNA · Translation · Nucleoside

Introduction

In a 1988 letter to the Editor of this journal, one of us proposed the hypothesis [1] that the multidrug-resistant (MDR) membrane protein (P-glycoprotein; P-gp) is responsible for the renal secretion of organic cations. At that time, the known substrates for P-gp were, principally, lipophilic organic cations, analogous to the known substrates for the renal organic cation transporter (OCT). Further, P-gp had been demonstrated in the apical membranes of secretory epithelia, particularly in the membranes lining the lumen of human proximal tubular cells [2], the site for postulated active OCT [3]. Subsequently as tests of this hypothesis, we compared the development of OCT and P-gp expression in neonate kidneys and found them to be temporally dissociated [4]. Additionally, membrane vesicles prepared from tumor cells exhibiting the classical MDR phenotype were shown not to enhance organic cation (tetraethylammonium; TEA) proton or TEA countertransport [5], features characteristic of OCT in renal brush border membrane vesicles [6,7]. Finally, although the basolateral-to-apical transepithelial flux of a classical MDR substrate, daunomycin, is enhanced in dog kidney distal tubule (MDCK) cells that have been transduced with the cDNA for human *MDR1* [8,9], it is not enhanced for a classical OCT substrate, 2'-deoxytubercidin (dTub) [10]. Herein, we present additional data that indicate the OCT and P-gp to be distinct entities using *Xenopus* oocytes as a functional translation-expression system for OCT and P-gp.

Materials and methods

Kidneys were removed and rapidly frozen in liquid nitrogen. Whole mouse kidney, pig kidney cortex or MDCK-MDR cells [8] were homogenized in guanidinium isothiocyanate, and RNA was collected by cesium chloride gradient centrifugation [11]. Poly A^+ RNA was isolated by two cycles of affinity chromatography on oligo-dT cellulose (Collaborative Research, Bedford, Mass) and checked before use by an in vitro rabbit reticulocyte translation system (Stratagene LaJolla, Calif.). Ovarian tissue was dissected from adult female *Xenopus laevis* (Nasco, Fort Atkin, Wis.) that were anesthetized with 3-aminobenzoic acid, ethyl ester. The ovaries were placed in modified Barth's solution (88 m*M* NaCl; 1 m*M* KCl; 2.4 m*M* $NaHC0_3$; 10 m*M* Hepes buffer, pH 7.5; 0.82 m*M* $MgSO_4$; 0.33 m*M* $Ca(NO_3)_2$; 0.41 m*M* $CaCl_2$; 10 μg/ml penicillin G and

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streptomycin). Manual defolliculation was performed on mature stage V and VI oocytes and healthy oocytes were injected with 30 ng poly A^+ RNA or an equal volume of water using 10 to 20 oocytes per data point. The oocytes were maintained at 18–20 °C for 5 days at which time transport assays were performed. The uptake of ^{3}H-dTub (15 Ci/mmol) or ^{3}H-vinblastine (9 Ci/mmol; Moravek Biochemicals, Brea, Calif.) was determined by incubating the oocytes as described above. The radioactivity in the oocytes was established by solubilizing them in 0.2 *N* NaOH plus 0.2% SDS and liquid scintillation counting. Alternatively, for direct measurement of dTub efflux, oocytes were injected with ^{3}H-dTub and the disappearance of radiolabel in the oocytes was determined as a function of time.

Results

A kidney secretory system localized to the apical membrane should manifest itself in the *Xenopus* oocyte translation system as a mRNA-dependent ability to maintain a lower internal level of transported solute, i.e. the rate of efflux should be enhanced in mRNA-injected oocytes compared to controls. Figure 1 illustrates the enhanced efflux of the OCT substrate, dTub, from oocytes that had been treated with mRNA from mouse kidneys compared to water-injected controls. Although not shown, similar results were observed in separate experiments in which the efflux was measured at 1 and 2 h. Additionally, the steady-state uptake (pmol/oocyte; 4 h) of dTub was reduced in oocytes injected with either mouse or pig kidney mRNA versus water-injected controls, i.e. 163 ± 15 vs 218 ± 26, mouse; 145 ± 19 vs. 193 ± 8, pig; mean $\pm$ SE; $P < 0.05$, $N = 5$–10 eggs each. Thus, the renal mRNA/*Xenopus* oocyte translation system demonstrates the anticipated transport of this OCT substrate.

In contrast to the results obtained using mouse and pig kidney mRNA that should express both OCT and P-gp, the mRNA from MDCK cells that overexpresses the human *MDR*1 P-gp did not enhance the efflux of dTub, further evidence that dTub is not a substrate for P-gp (Fig. 2). The mRNA used for this experiment was shown to be functional since it did reduce the steady-state uptake of the classical MDR substrate, vinblastine.

Discussion

The *Xenopus* oocyte translation system has been used extensively by others in the functional cloning of a number of membrane transport proteins [12–14]. In most cases, the transporters facilitate the inwardly directed flux of solute, and the solutes have frequently been relatively excluded from the oocyte in the absence of expressed transporter. With regard to mammalian kidney, expression of a transporter for renal reabsorption via the apical membrane should manifest itself as a mRNA-dependent, enhanced uptake of substrate into the oocytes. Indeed, this is the case with the sodium-glucose symporter [12] and sodium-coupled

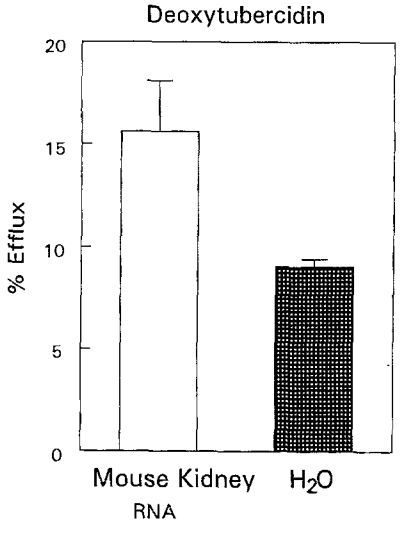

Fig. 1 Efflux of dTub from *Xenopus* oocytes after injection of total mRNA from adult mouse kidney. mRNA (30 ng) or an equivalent volume of water was injected into the oocytes on day 0. Radioactive dTub (16 pmol; 16 nCi) was then injected on day 5 and the percentage of total isotope effluxed was determined after a 1.5 hr incubation period as described in Materials and methods. The results shown are from an experiment that employed 10–15 oocytes per group, mean values $\pm$ SE are given. This experiment is representative of seven separate experiments performed with three different mouse kidney mRNA preparations

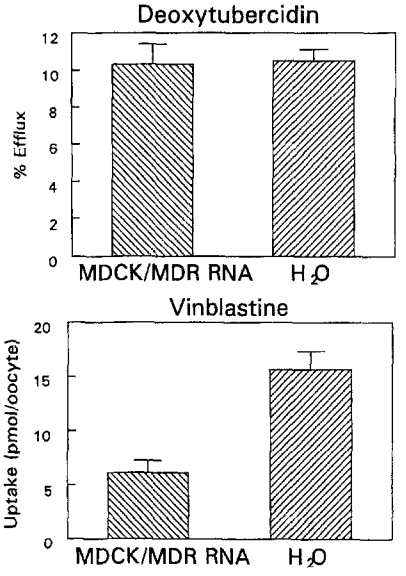

Fig. 2 Failure of P-glycoprotein to enhance the rate of dTub efflux from *Xenopus* oocytes. Experiments were performed as described in the legend to Figure 1 using mRNA prepared from the P-glycoprotein over expressing cell line, MDCK-MDR (dog distal tubule cells transduced with the human cDNA for *MDR1*). The efflux of dTub was measured as described in the legend to Figure 1, whereas the "steady-state" vinblastine uptake was determined after 1 h incubation in Barth's medium containing 100 μM ^{3}H-vinblastine. Results shown are mean values $\pm$ SE, $n = 10$–15

nucleoside transporters [14] that may be involved in renal reabsorption. With regard to carriers that facilitate the renal secretion of solutes at the apical membrane, the enhanced diffusion should be outwardly directed in the oocytes, resulting in a lower steady-state level within the oocytes. Experiments in which mouse and pig kidney mRNA were injected into the oocytes suggest that this is the case for the OCT substrate, dTub (Fig. 1). Since the mRNA for P-gp should be present in these samples, it was important to utilize a cell that should not express OCT. MDCK cells should not be capable of expressing OCT since they are derived from a distal tubule and OCT is a feature of proximal but not distal tubules. Indeed, mRNA from MDCK cells that overexpressed P-gp were incapable of enhancing the efflux of dTub from injected oocytes (Fig. 2). The observation that the same mRNA preparation did lower the steady-state level of vinblastine in the oocytes indicated the mRNA to be functional. Functional expression of P-gp in *Xenopus* oocytes has previously been demonstrated by others using in vitro transcribed pure cDNA, yielding results with vinblastine similar to those shown in Fig. 2 [15].

In addition to glomerular filtration, human [16] and mouse [17] kidneys secrete 2'-deoxyadenosine. Using dTub as a nonmetabolized and nontoxic deoxyadenosine analog to study the mechanism(s) for deoxynucleoside secretion, we have demonstrated its renal secretion in mice to occur by the classical OCT using a number of in vitro [17] and in vivo [18] methods. dTub is not a substrate for P-gp since its flux accross MDCK cells is not influenced by levels of P-gp expression [10]. Also, the toxicity of dTub analogs (nucleosides) is not altered by the expression of MDR [10]. Thus, the data indicate that dTub (and perhaps TEA, NMN, deoxyadenosine) renal secretion occurs by OCT, a mechanism distinct from P-gp. Classical OCT is characterized by organic cation-proton and organic cation-organic cation exchange in brush border membrane vesicles [6,7]. The uptake of TEA (a classical OCT substrate) was not enhanced in membrane vesicles prepared from MDR tumor cells, and the vesicles failed to demonstrate cation-proton or cation-cation exchange under conditions in which such exchange has been demonstrated for pig kidney cortex brush border membrane vesicles [5]. It is now reasonable to conclude that OCT and P-gp are different carriers, one (P-gp) for which transport is energized by ATP hydrolysis and the other (OCT) by proton exchange. The fact that P-gp resides in the apical membrane of proximal tubule cells strongly suggests a role in the renal secretion of some organic cations [9,19] and perhaps other P-gp substrates. Nonetheless, the data presented and discussed herein indicate P-gp and OCT to be distinct.

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