Expression of renal organic cation transporter in *Xenopus laevis* oocytes

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The expression of the organic cation transport system of rat renal proximal tubules has been studied in *Xenopus laevis* oocytes injected with poly(A)⁺ RNA from the rat renal cortex. The effectiveness of the technique was confirmed by examining expression of the Na⁺/D-glucose co-transporter. Compared with water-injected and non-injected oocytes, the injection of total poly(A)⁺ RNA resulted in about a 3-fold increase in tetraethylammonium (TEA) uptake activity. TEA uptake by poly(A)⁺-RNA-injected oocytes was time-dependent and was inhibited by cimetidine and HgCl₂, but not by *p*-aminohippurate. After size-fractionation on a sucrose density gradient, a 1.4–2.4 kb poly(A)⁺ RNA fragment was identified that expressed the organic cation transport system in oocytes. These results demonstrate that the renal organic cation transporter was expressed in oocytes and that this expression system can provide an effective assay procedure for cloning of the organic cation transporter.

INTRODUCTION

The kidney has the function of excreting a large number of foreign compounds. Many of these compounds undergo filtration in the glomeruli and net secretion in the proximal tubules. It is well known that organic cations are actively secreted from plasma to filtrate by a specifically mediated pathway. Using isolated membrane vesicles, we [1–4] and others [5–9] have reported that organic cations are transported by carrier-mediated processes in both brush-border and basolateral membranes. In brush-border membranes, various compounds such as tetraethylammonium (TEA) [1,10–12], aminocephalosporins [13], cimetidine [2,9,14] and N¹-methylnicotinamide [6,15,16] are transported by an outward H⁺ gradient as a driving force (H⁺/organic-cation antiport system). In basolateral membranes, organic cations are transported via an inside-negative membrane potential [1,8] and/or an exchange mechanism [17,18] as the driving force. Additionally, thiol groups [6,10], carboxyl groups [15] and histidine residues [12] are reportedly essential for organic cation transport in renal brush-border membranes, using chemical modifiers for amino acid residues.

To further characterize the nature of the organic cation transporter, it is necessary to isolate the transporter protein and/or to clone the gene encoding it. However, there have been no reports identifying the molecular structure of the organic cation transporter. Recent developments in the methodology for membrane protein expression in *Xenopus laevis* oocytes injected with exogenous poly(A)⁺ RNA have resulted in new approaches to cloning of the transporters without antibodies or oligonucleotide probes [19]. Since expression and cloning of the intestinal Na⁺/D-glucose co-transporter was reported by Hediger *et al.* [20,21], transport proteins for various endogenous substances, such as amino acids [22–24], bile acids [25] and dipeptides [26] have been expressed in *Xenopus laevis* oocytes injected with poly(A)⁺ RNA. In the present report, we demonstrate the functional expression of the organic cation transporter, which plays an important role in the renal secretion of organic cations, in oocytes injected with poly(A)⁺ RNA from the rat renal cortex.

EXPERIMENTAL

Materials

[1-¹⁴C]TEA (4 mCi/mmol) and [¹⁴C]α-methyl D-glucoside (100 mCi/mmol) were obtained from Du Pont–New England Nuclear (Boston, MA, U.S.A.). Oligo(dT)–cellulose was obtained from Collaborative Research Inc. (Bedford, MA, U.S.A.). Guanidinium thiocyanate, caesium chloride, sucrose and Hepes were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Collagenase was purchased from Wako Pure Chemicals (Osaka, Japan). All other chemicals used were of the highest purity available.

Oocyte isolation

Small pieces of ovarian lobes were dissected from cold-anaesthetized *Xenopus laevis* (TOPS, Tokyo, Japan) and shaken gently at 20 °C for 90 min in a solution consisting of 88 mM-NaCl, 1 mM-KCl, 1 mM-MgSO₄, 2.4 mM-NaHCO₃ and 7.5 mM-Tris/HCl, pH 7.6, containing 1 mg of collagenase/ml. Denuded healthy-looking oocytes, 1 mm in diameter, were selected and injected with 50 nl of water or of a poly(A)⁺ RNA solution (1–1.4 ng/nl)/cell. The oocytes were maintained at 20 °C for 4 days in sterile Barth's saline consisting of 88 mM-NaCl, 1 mM-KCl, 1 mM-MgSO₄, 2.4 mM-NaHCO₃ and 7.5 mM-Tris/HCl, pH 7.6, supplemented with 10 units of penicillin/ml and 10 µg of streptomycin/ml.

RNA isolation and size fractionation

Kidneys of male Wistar albino rats were removed and the renal cortex was separated from the medulla. Tissues were homogenized in 20 vol. of extraction buffer consisting of 4.2 M-guanidinium thiocyanate, 25 mM-sodium citrate and 0.1 mM-2-mercaptoethanol, and then total cellular RNA was isolated by caesium chloride centrifugation. Poly(A)⁺ RNA was selected by oligo(dT)–cellulose chromatography. For size fractionation, total poly(A)⁺ RNA samples (250 µg) were heated at 65 °C for 5 min, then applied to a 5–25% (w/w) sucrose gradient containing 10 mM-Tris/HCl, pH 8.0, 2 mM-EDTA and 0.5% SDS. The gradient was centrifuged at 15000 *g* for 16 h at 20 °C and subsequently divided into 80 × 0.4 ml fractions.

Abbreviation used: TEA, tetraethylammonium.

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Size-fractionated mRNA was electrophoresed on a 1% agarose/0.66 M-formaldehyde gel, followed by ethidium bromide staining.

Assay of transport activity

TEA and α -methyl D-glucoside uptake into oocytes was measured using a radiotracer. In brief, 5–7 oocytes were incubated at 25 °C for the indicated times in Frog Ringer's solution consisting of 105 mM-NaCl, 2.5 mM-KCl, 1.8 mM-CaCl₂ and 10 mM-Hepes/NaOH, pH 7.4, containing 1 mM-[¹⁴C]TEA or 1 mM-[¹⁴C] α -methyl D-glucoside. Incubation was terminated by washing the oocytes with 3 × 2 ml of ice-cold Frog Ringer's solution. Each oocyte was dissolved in 0.2 ml of 0.2 M-NaOH containing 0.2 %

Fig. 1. Time course of TEA uptake into poly(A)⁺-RNA-injected oocytes

Oocytes were injected with 50 ng of total poly(A)⁺ RNA (injection volume of 50 nl) or the same volume of water. After 4 days of culture at 22 °C, the uptake study was performed. The concentration of TEA was 1 mM. Five oocytes were used for each determination. ●, Poly(A)⁺-RNA-injected oocytes; ○, water-injected oocytes. The uptake values are means ± S.E.M. from 15 determinations in three separate oocyte preparations.

Table 1. Effect of cimetidine, HgCl₂ and *p*-aminohippurate on the activity of the expressed transporter

Uptake of 1 mM-TEA in water-injected oocytes and poly(A)⁺ RNA-injected oocytes was measured at 25 °C for 1 h. The composition of the uptake medium was Frog Ringer's solution (as described in the Experimental section), in either the presence (+) or the absence (–) of cimetidine (5 mM), HgCl₂ (0.1 mM) or *p*-aminohippurate (PAH, 5 mM). Values are means ± S.E.M. from 15 measurements in three separate oocyte preparations. **P* < 0.01 by Student's *t* test, compared with that in the absence of the additive.

Addition		TEA uptake (pmol/h per oocyte)	
		Water-injected	Poly(A) ⁺ RNA-injected
Cimetidine	–	13.98 ± 1.58	39.01 ± 4.22
	+	19.38 ± 3.09	17.99 ± 3.52*
HgCl ₂	–	8.92 ± 2.57	38.71 ± 1.19
	+	14.09 ± 0.55	20.78 ± 4.74*
PAH	–	11.05 ± 1.82	48.17 ± 2.50
	+	9.87 ± 4.49	41.12 ± 6.30

Fig. 2. Expression of TEA uptake with fractionated poly(A)⁺ RNA

Fractionation of the poly(A)⁺ RNA on a linear sucrose density gradient. Total poly(A)⁺ RNA (250 µg) was size-fractionated on a linear 5–25% (w/w) sucrose density gradient. After centrifugation, 80 × 0.4 ml fractions were collected and poly(A)⁺ RNA from each fraction was precipitated, washed and resuspended in water (RNA concentrations were approx. 0.8 ng/nl). Uptake of 1 mM-TEA was measured at 25 °C for 1 h. The uptake values represent means ± S.E.M. from 10 determinations in two separate oocyte preparations.

SDS and the radioactivity was assayed by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

The expression of renal organic cation transporter activity was monitored by measuring [¹⁴C]TEA uptake by *Xenopus* oocytes that had been injected with poly(A)⁺ RNA from the rat renal cortex. Oocytes injected with water or nothing both exhibited low TEA uptake. The rates of TEA uptake by non- and water-injected oocytes were 8.36 ± 2.94 pmol/h per oocyte and 9.61 ± 3.03 pmol/h per oocyte respectively, which increased by nearly 3.3-fold to 28.95 ± 3.65 pmol/h per oocyte in poly(A)⁺-RNA-injected oocytes (values are means ± S.E.M. from 30 determinations with six separate preparations).

We also measured [¹⁴C] α -methyl D-glucoside uptake by the poly(A)⁺-RNA-injected oocytes, because the Na⁺/D-glucose co-transporter is located in the brush-border membranes of intestinal and renal epithelial cells, and glucose uptake should indicate the effectiveness of the expression technique as the necessary control. The value for α -methyl D-glucoside uptake was 7.71 ± 0.98 pmol/h per oocyte in water-injected oocytes. Uptake was enhanced about 3-fold, to 26.50 ± 2.73 pmol/h per oocyte, in oocytes injected with poly(A)⁺ RNA from the rat renal cortex, which demonstrated that the procedures used for expression and uptake were effective (means ± S.E.M., *n* = 30).

The time course of TEA uptake by oocytes was examined. As shown in Fig. 1, uptake of TEA was low in water-injected oocytes and did not significantly increase with time. TEA uptake by oocytes injected with renal cortical poly(A)⁺ RNA, however, greatly increased with the length of the incubation.

Table 1 shows the effects of the organic cation transport inhibitors cimetidine and HgCl₂ [1,2], and the organic anion *p*-aminohippurate, on TEA uptake. TEA uptake expressed in poly(A)⁺-RNA-injected oocytes was greatly inhibited by cimetidine and HgCl₂. On the other hand, uptake by water-injected oocytes did not significantly change in the presence of the two compounds. In addition, no inhibition of TEA uptake was observed with *p*-aminohippurate. These results suggest that the inhibitory effect of cimetidine and HgCl₂ was not non-specific interference, and that the expressed protein has similar characteristics to that of the renal organic cation transporters. TEA uptake by water-injected oocytes may reflect passive, non-

Fig. 3. Representative formaldehyde gel electrophoretogram of fractionated poly(A)⁺ RNA

Aliquots (3 µg) from several of the size-selected poly(A)⁺ RNA fractions were electrophoresed in a denaturing agarose gel. Molecular standards are shown on the right (M). From the size distribution of fractions 50 and 55, the size of poly(A)⁺ RNA in fraction 51 was estimated to range between 1.4 and 2.4 kb. The prominent bands in fraction 30 and in fractions 50 and 55 are 28 S and 18 S ribosomal RNAs respectively.

mediated diffusion. Alternatively, *Xenopus* oocytes possess an endogenous TEA transporter that may be distinct from the renal organic cation transport system.

The poly(A)⁺ RNA fractions which translate the TEA transport protein were identified by injecting poly(A)⁺ RNA from representative fractions obtained from sucrose density gradient centrifugation. As shown in Fig. 2, the greatest TEA uptake was seen following the injection of poly(A)⁺ RNA from fraction 51. The size of fraction 51 poly(A)⁺ RNA was determined using formaldehyde gel electrophoresis (Fig. 3). The size range of the poly(A)⁺ RNA that resulted in maximal TEA uptake was approx. 1.4–2.4 kb.

TEA transport in renal brush-border membranes, but not in basolateral membranes, is driven by an outward H⁺ gradient [1,3,7,8]. Therefore, in order to specify the type of organic cation transporter expressed in the oocytes, uptake was studied in the presence of an outward H⁺ gradient. Oocytes incubated in Frog Ringer's solution (pH 8.0) showed only a slight increase of TEA uptake (approx. 1.1-fold) compared with those under conditions of no H⁺ gradient. Thus the transporter expressed in poly(A)⁺-RNA-injected oocytes may represent that in the basolateral membranes. However, this may not be a conclusive evidence for lack of a H⁺/TEA antiport system, because a sufficient H⁺ gradient may not be maintained in the present experimental conditions (the internal pH was not monitored). Moreover, TEA transport in brush-border membranes is affected not only by the proton gradient (a driving force) but also by pH itself (optimum pH 7.0) [11], and therefore the effect of a H⁺ gradient may be difficult to detect in the present experimental conditions. In addition, it cannot be ruled out that both transport systems are simultaneously expressed in the oocyte. Further studies are needed to clarify these issues.

TEA might be suspected to inhibit slowly-activated K⁺ channels [27] which may also be expressed after injection of the rat kidney poly(A)⁺ RNA. However, when the oocytes were voltage-

clamped at -60 mV, no current response was observed by a bath-application of 10 mM-TEA (results not shown). As the resting potential of poly(A)⁺-RNA-injected oocytes was about -50 mV, the observed TEA uptake by the oocytes was not due to the interaction of TEA with K⁺ channels.

In conclusion, the present study demonstrates that injection of rat renal cortical poly(A)⁺ RNA into *Xenopus laevis* oocytes results in the functional expression of organic cation transport activity. This expression system represents a promising approach to cloning the gene and to the further characterization of the molecular properties of the renal organic cation transporter.

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