2006

Organic Anion and Cation Transporter Expression and Function During Embryonic Kidney Development and in Organ Culture Model Systems

Douglas H. Sweet
Virginia Commonwealth University, dsweet@vcu.edu

Satish A. Eraly
University of California - San Diego

Kevin T. Bush
University of California - San Diego

Sanjay K. Nigam
University of California - San Diego

Copyright 2006 International Society of Nephrology. This is the author's manuscript of a work that was accepted for publication in Kidney Int. 2006 Mar; 69(5): 837–845. The final publication is available at http://dx.doi.org/10.1038/sj.ki.5000170

Published in final edited form as:
Kidney Int. 2006 March ; 69(5): 837–845. doi:10.1038/sj.ki.5000170.

Organic Anion and Cation Transporter Expression and Function During Embryonic Kidney Development and in Organ Culture Model Systems

Douglas H. Sweet[b,ξ], **Satish A. Eraly**[b], **Kevin T. Bush**[b], and **Sanjay K. Nigam**[a,b,c]

[a]Department of Pediatrics, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0693

[b]Department of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0693

[c]Department of Cellular and Molecular Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0693

Abstract

Background—Organic anion and cation transporters (OATs, OCTs and OCTNs) mediate the proximal tubular secretion of numerous clinically important compounds, including various commonly prescribed pharmaceuticals. Here, we examine the ontogeny of these transporters in rat embryonic kidney in detail, both *in vivo* and in two *in vitro* organ culture models of kidney development, whole embryonic kidney (WEK) culture and culture of induced metanephric mesenchyme (MM).

Methods—We used QPCR to determine expression levels of transporter genes in rat embryonic kidneys on each day of gestation from ed13 to ed18, in induced and un-induced MM, and on each day of one week of WEK culture. We also used uptake of fluorescein as a novel functional assay of organic anion transporter expression in WEK and MM.

Results—The developmental induction of the various organic anion and cation transporter genes does not occur uniformly: some genes are induced early (e.g., Oat1 and Oat3, potential early markers of proximal tubulogenesis), and others not till kidney development is relatively advanced (e.g., Oct1, a potential marker of terminal differentiation). We also find that the ontogeny of transporter genes in WEK and MM is similar to that observed *in vivo*, indicating that these organ culture systems may appropriately model the expression of OATs, OCTs and OCTNs.

Conclusion—We show that WEK and MM cultures may represent convenient *in vitro* models for study of the developmental induction of organic anion and cation transporters. Functional organic anion transport as measured by fluorescein uptake was evident by accumulation of the fluorescence in the developing tubule in these organ cultures. By demonstrating the mediated uptake of fluorescein in WEK and MM, we have established a novel *in vitro* functional assay of transporter function. We find that OATs, OCTs, and OCTNs are differentially expressed during proximal tubule development. Our findings on the renal ontogeny of organic anion and cation transporters could carry implications both for the development of more rational therapeutics for premature infants, as well as for our understanding of proximal tubule differentiation.

Address correspondence to: Douglas H. Sweet Department of Pharmaceutical Sciences Medical University of South Carolina 280 Calhoun Street (Room QE218) PO Box 250140 Charleston, SC 29425 Phone: (843)-792-2348 Fax: (843)-792-1712 sweetd@musc.edu.
[ξ]See acknowledgements for current address.

Keywords

organic anion transporter (OAT); organic cation transporter (OCT); ontogeny; embryonic kidney; organ culture; whole embryonic kidney (WEK); metanephric mesenchyme (MM); functional assay

Many organic ions are excreted from the body via secretion mediated by specific transporters in the proximal tubule of the kidney. Such transporters are located on either the basolateral or apical surface of the proximal tubular cell, positioned so as to potentially mediate the successive steps of basolateral uptake and apical efflux that are required for tubular secretion. Individual transporters are also (broadly) specific for either organic anions or organic cations. Accordingly, four functional groups of these transporters might be distinguished, apical or basolateral organic anion transporters (OATs), and apical or basolateral organic cation transporters (OCTs/OCTNs). Notwithstanding these functional distinctions, OATs and OCTs/OCTNs are in fact phylogenetically related (as evidenced by their structural homology), and are accordingly grouped within the same gene family (slc22 [1], in turn grouped within the Major Facilitator Superfamily [MFS] of solute carriers [2]).

These transporters are of considerable pharmacological and physiological interest, because their substrates include numerous commonly used drugs (encompassing antibiotics, antivirals, diuretics, non-steroidal anti-inflammatories, H2-blockers, and antihypertensives, among others). In addition, several physiologically important endogenous compounds are also substrates; these include urate, folate, prostaglandins, choline, and monoamine neurotransmitters (for recent reviews, see [3-5]). Thus investigation of the ontogeny of OATs, OCTs and OCTNs will have significant implications for our understanding of the developmental maturation of the body's capacity to ‘handle’ many clinically important substances.

Following the molecular cloning of the genes encoding organic anion and cation transporters (reviewed in [3,4]), there have been several studies of the developmental changes in expression of these genes in the early post-natal period [6-8]. By contrast, changes in gene expression occurring during embryogenesis have not been studied in detail. We previously investigated the embryonic expression of Oat1-3 and Oct1 in mouse [9]. We discovered that renal expression of these genes (as determined by *in situ* hybridization and Northern blotting) is detectable as early as ed14, approximately coinciding with the onset of proximal tubular differentiation. (We also discovered, unexpectedly, that these genes manifest transient embryonic expression in a variety of disparate tissues including brain, spinal cord, aorta, heart, lung, liver, and bone, in addition to kidney. These observations suggest a possible role for the encoded transporters in development, conceivably involving transport of morphogens.)

A more careful analysis not only has implications for understanding the basic biology of proximal tubule differentiation, but may also bear on the ability of premature infants to handle a variety of drugs. We have now performed a detailed examination of the renal ontogeny of organic anion and cation transporters in rat. The specific genes examined were Oat1 (*slc22a6*; [10-13]), Oat2 (*slc22a7*; [14,15]), Oat3 (*slc22a8*; [16,17]), Oct1 (*slc22a1*; [18,19]), Oct2 (*slc22a2*; [20,21]), OCTN1 (*slc22a4*; [22]), and OCTN2 (*slc22a5*; [23-25]). Expression of the apical proximal tubular organic anion transporters, OAT4 (*slc22a11*; [26]) and RST/URAT1 (*slc22a12*; [27,28]), could not be examined because the corresponding rat orthologs, if they exist, have not yet been identified. We used QPCR to determine expression levels of the above genes in rat embryonic kidneys on each day of gestation from ed13 to ed18, correlating our findings with expression levels of genes encoding other markers of proximal tubular differentiation, NaP_i2 (Na^+/phosphate co-transporter [29]) and SGLT1 (Na^+/glucose co-transporter [30]). We also examined the expression of organic anion and cation transporters

in two *in vitro* models of kidney development, whole embryonic kidney (WEK) culture [31] and co-culture of metanephric mesenchyme (MM) with spinal cord [32]. We determined transporter expression in these systems both by QPCR as well as functionally, by assessment of transport of the fluorescent compound, fluorescein, which is a specific OAT substrate [33-35]. We found that the ontogeny of transporter genes in either *in vitro* model was similar to that observed *in vivo*. Therefore, WEK and MM cultures may represent convenient *in vitro* models for study of function as well as of expression of organic anion and cation transporter genes.

METHODS

Culture of Whole Embryonic Kidney

Uteri from timed pregnant Sprague-Dawley rats at day 13 of gestation (day 0 of gestation being the day of appearance of the vaginal plug) were removed and the embryos dissected free of any surrounding membranes and tissue. Kidneys were isolated from the embryos and placed on polycarbonate Transwell filters with no separation of males and females. The organ cultures were maintained in DME/F12 (MediaTech, Herndon, VA) supplemented with 10% FBS up to 7 days.

Isolation and culture of metanephric mesenchyme

Embryonic kidneys, isolated as described above, were incubated in L-15 media containing 0.1% trypsin and 0.05 U/μl DNase I for 15 min 37°C (no separation of males and females). Trypsinization was stopped by the addition of FBS (final concentration of 10%) and the metanephric mesenchyme was dissected free of the ureteric bud using minutia pins. Pieces of embryonic spinal cord were also dissected free of surrounding tissues and collected for use as the heterologous inducer of the MM. Embryonic spinal cord was applied to the top of a polycarbonate Transwell filter and isolated MM was placed in contact with the embryonic spinal cord. Tissues were maintained in DME/F12 (MediaTech, Herndon, VA) supplemented with 10% FBS. After 24 hr of induction the spinal cord tissue was removed and the induced MM cultured for an additional 5 days.

Quantitative polymerase chain reaction

All RNA samples were isolated using the Absolutely RNA RT-PCR Miniprep Kit (Stratagene, La Jolla, CA) with inclusion of the optional DNase I digestion step in the protocol. RNA isolated from adult rat kidney was reverse transcribed and used as template for standard curve reactions for each primer pair. Standard curve values were obtained for 0.01 ng, 0.1 ng, 1 ng, and 10 ng dilutions of the original amount of RNA that was reverse transcribed. No-template control reactions for each primer pair were incorporated into each QPCR run. Primer pair sequences used for QPCR analysis are listed in Table 1, final concentration in QPCR reactions was 50 nM. Reactions were set up in 96 well plates using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) according to manufacturer's specifications and samples were run on an ABI Prism 7700 sequence detection system at the UCSD QPCR core facility. Cycle parameters were as follows: initial 95°C denaturation step for 10 min followed by 40 cycles of 95°C denature for 20 s, 60°C anneal for 20 s, and 72°C extension for 45 s. The data were analyzed using Sequence Detector v1.7 software. Data were normalized to the level of expression of GAPDH in the same sample, and are reported as the ratio of the level of normalized gene expression in the sample of interest to the level of gene expression in adult kidney (mean value ± SD; embryonic kidney and MM were triplicate samples; cultured kidney, duplicate samples).

Sample tissues were obtained as follows: Embryonic kidneys: renal masses were directly dissected from ed13 through ed18 fetal rats and pooled for RNA isolation; Cultured kidneys:

renal masses were dissected from ed13 fetal rats, cultured on Transwell support membranes for 1 to 7 days (as described above in embryonic kidney culture), and RNA isolated from pooled tissue; Induced metanephric mesenchyme: pooled MM tissue isolated from 13-16 ed13 rat fetuses was co-cultured with ed13 spinal cord for 24 hr and then cultured for an additional 5 days after the removal of the spinal cord tissue (as described above in metanephric mesenchyme culture), RNA from the induced MM was then isolated from pooled tissue.

Each RNA sample was reverse transcribed using oligo-dT and Moloney Murine Leukemia Virus Reverse Transcriptase (Promega Corporation, Madison, WI) in reactions containing 2 μg of RNA per 25 μl of reaction volume. Each QPCR reaction contained 1 μl of RT reaction as template with the exception of the ed18 kidney samples for which the template was diluted 10 fold. Standard curves and all samples to be compared (*e.g.*, ed13 through ed18 or kidney cultured for 1 day through 7 days) were analyzed on the same QPCR plate. With the exception of β-actin in all systems, and Oat2, Oct1, and NaP_i2 in the induced MM cultures, the expression of each gene was determined in samples prepared from two independent experiments (*i.e.*, run 1 and run 2).

Fluorescein uptake assays

Embryonic day 13 kidneys cultured for 7 days or induced MM cultured for 6 days were exposed to 2 μM fluorescein (FL) in the culture medium (with phenol red, antibiotics, and serum omitted) for 30 min in the absence or presence of 2 mM probenecid. Following the uptake period, the organ cultures were washed twice with ice-cold phosphate-buffered saline prior to examination by confocal fluorescence microscopy. Prior to confocal microscopy of experimental groups, control tissues (no exposure to FL) were examined and microscope levels set such that no background fluorescence was detectable.

RESULTS

Transporter expression during embryonic kidney development

The expression profiles of 10 genes (Oat1, Oat2, Oat3, Oct1, Oct2, OCTN1, OCTN2, SGLT1, NaP_i2, and β-actin) during rat embryonic kidney development from ed13 through ed18 were examined by QPCR. Standard curves were generated for each gene of interest using a dilution series of reverse transcribed adult rat kidney RNA as template. Profiles were then generated for each gene of interest in the various tissue samples and values for their respective levels of expression extrapolated from the standard curves (Fig. 1). The level of expression of each gene of interest was then normalized to the level of expression of GAPDH in the same tissue sample (Fig. 2).

Of all the genes examined, only Oat2 expression was never detected in embryonic kidney. Since Oat2 message was readily detectable in newborn and adult rat kidney (not shown), its expression is likely to be upregulated between ed18 and birth. The expression patterns of the other genes fell into three groups: Group 1 genes manifested a generally progressive increase between ed13 and ed18; these included the organic anion transporters Oat1 and Oat3, and the proximal tubular markers SGLT1 and NaP_i2. Group 2 genes manifested by and large similar expression over the course of embryonic development. These genes included the organic cation transporter, Oct2, and the organic cation/carnitine transporters, OCTN1 and OCTN2, and, as expected, the housekeeping gene, β-actin. Finally, Oct1 was grouped separately as Group 3 since it manifested a sharp rise in expression relatively late in kidney development, around ed18.

Transporter expression in the induced metanephric mesenchyme culture model

Culture of the metanephric mesenchyme (MM; the embryonic tissue which is destined to give rise to the nephrons of the kidney) in the presence of a suitable inducer has long been used to model nephrogenesis *in vitro* [31,32]. For example, following induction by embryonic spinal cord, cultured MM forms proximal tubule-like structures. We cultured spinal cord-induced and un-induced MM (Fig. 3; see *Methods* for description of culture and induction of MM), and determined the expression of organic anion and cation transporter genes (as above, Oat1, Oat2, Oat3, Oct1, Oct2, OCTN1, OCTN2, SGLT1, and NaP_i2) and of the control gene, β-actin, in the two culture systems (Fig. 4). The expression of all the transporter genes, with the sole exception of OCTN2, was substantially upregulated by spinal cord induction (Group A in Fig. 4). Expression of OCTN2 was similar in the induced and un-induced cultures, as was that of the control β-actin gene (Group B).

Organic anion transport function in the induced metanephric mesenchyme culture model

We demonstrated the presence of functional organic anion transport activity in induced MM by observing the inhibitor-sensitive uptake of the OAT substrate fluorescein (FL), which appears to be a specific substrate for Oat1 and Oat3 [33-35] (Fig. 5). FL uptake was completely blocked by pre-exposing the tissue to 2 mM probenecid or 10 mM *p*-aminohippurate for 1 hr prior to the addition of FL to the culture medium (data not shown). Moreover, FL accumulation occurred in a highly specific pattern being associated only with the induced tubule-like structures (Fig. 5B). Observation at higher magnification revealed that the induced tubule-like structures appeared to consist of a single layer of cells arranged around a central fluid-filled space or lumen (Fig. 5C & E). In correlation with this interpretation, FL was observed to be concentrated within this space (Fig. 5D & F).

Transporter expression in the cultured kidney model

Culture of the whole embryonic kidney (derived from ed13 rat embryos) is a second *in vitro* system that is commonly used to model kidney development (Fig. 6). We used QPCR to assess expression of Oat1, Oat2, Oat3, Oct1, Oct2, OCTN1, OCTN2, SGLT1, NaP_i2, and β-actin in WEK cultures (Fig. 7). Two major patterns of gene expression could be discriminated: Oat1, Oat3, Oct1, SGLT1, and NaP_i2 manifested a progressive increase in expression over the seven days of WEK culture (Group A), while Oct2, OCTN1, OCTN2, and β-actin expression did not substantially change over the duration of culture (Group B). Oat2, which was not detected in embryonic kidneys *in vivo* (Fig. 2), was also undetectable in WEK culture.

Organic anion transport function in the cultured kidney model

As with induced MM culture, we established the presence of organic anion transport in WEK (cultured for one week) by demonstrating accumulation of FL in convoluted tubule-like structures within the organ culture (Fig. 8B, arrows). These structures appeared to be continuous with a more deeply situated network of broader and straighter passages, presumably part of the collecting duct system (Fig. 8B, arrowheads). As observed with MM, accumulation of FL in WEK was completely inhibited by pretreatment with probenecid or *p*-aminohippurate (data not shown).

DISCUSSION

We have closely examined the ontogeny of organic anion and cation transporter expression during rat embryonic kidney development *in vivo*, using the highly sensitive method of QPCR. We find that Oat1 and Oat3, the principal basolateral organic anion transporters of the proximal tubule [11-13,16,17,36], as well as the proximal tubular markers, SGLT1 and NaP_i2 [29,30], manifest a progressive increase in gene expression over the period examined, ed13-18 (Fig.

2). Thus these genes might be considered relatively early markers of nephrogenesis (provided a sufficiently sensitive method of detection is employed). Of note, Oat1 and Oat3 are chromosomally paired and have similar expression patterns in adult tissue [1,37]. Therefore, their approximately cotemporal activation of expression during embryonic kidney development may represent an instance of coordinate transcriptional regulation of linked genes.

In contrast to the basolateral OATs, expression of the basolateral organic cation transporter, Oct1 [38,39], is undetectable till kidney development is fairly advanced, and therefore appears to be a marker of terminal differentiation. Oct2, the other basolateral organic cation transporter [38,39], and OCTN1 & OCTN2, apically located organic cation and carnitine transporters [40,41], do not manifest substantial changes in expression level over the period of embryonic development studied. Finally, Oat2 message, while expressed in adult rat and mouse kidney, was undetectable at any embryonic time point examined in this study (ed13 through ed18). These findings are consistent with a recent study examining OAT mRNA expression levels in rat kidney starting at birth, in which no appreciable expression of renal Oat2 mRNA was observed as late as 35 days of age [6].

Just as the prior studies of transporter expression in the neonatal period [6-8] could have implications for drug dosing in pediatric patients, our results could ultimately contribute to the development of more rational therapeutics for premature infants. Moreover, identification of individual OATs and OCTs as potential early (Oat1, Oat3) or late (Oct1) markers of proximal tubular differentiation could have significant implications for our understanding of the molecular mechanisms underlying this process. For example, expression of “early” genes could be under the direct control of “master” factors that are key regulators of proximal tubulogenesis. Indeed, analysis of the upstream regions of the Oat1 and Oat3 genes has revealed the presence of evolutionarily conserved binding sites for several transcription factors of demonstrated importance to kidney development, including PAX1, WT1, and HNF1, among others [37]. Of these factors, HNF1 seems particularly likely to be significantly involved in the maturation of the proximal tubule; it has been shown to induce the transcription of several proximal tubular transporters (including, of the ones examined in this study, SGLT1) [42-44], and its knockout results in a renal Fanconi syndrome with urinary loss of glucose, amino-acids, and phosphates [45].

We have also examined transporter expression and function in two prominent *in vitro* models of nephrogenesis: whole embryonic kidney (WEK) culture, and culture of induced metanephric mesenchyme (MM). The “organs” in these model systems grow and apparently differentiate during culture, with formation of tubules that bear a remarkable functional as well as structural resemblance to developing nephrons *in vivo* [31,32]. We now find that expression patterns of OATs, OCTs, and OCTNs in these organ cultures also closely resemble those observed *in vivo*.

In the MM model, we find that all of the genes whose expression increases at some point during *in vivo* nephrogenesis (*i.e.*, Oat1, Oat3, and OCT1) are also up-regulated in induced MM (Figs. 2 & 4). However, there is not an exact correspondence between induced MM and *in vivo* development, since the gene expression of Oct2 and OCTN1 appears to be upregulated in induced MM, but these genes do not manifest a clear trend toward increasing expression *in vivo*. A unique feature of the induced MM model is the striking upregulation of Oat2 expression; the only gene examined whose expression was undetectable at any time in either embryonic kidney or WEK culture. The WEK model recapitulates organic anion and cation transporter gene expression patterns to an even closer degree (Figs. 2 & 7): all the genes whose expression increases during *in vivo* nephrogenesis, but none of the genes whose expression does not, manifest increasing expression over the course of WEK culture.

Therefore, our results demonstrate that WEK and MM culture may represent convenient *in vitro* models for study of the developmental induction of organic anion and cation transporter gene expression. In addition, our results demonstrate mediated transport of the OAT-substrate fluorescein in these models. This latter finding suggests that the transporter genes expressed during embryonic development are, at least in some instances, functional. It also establishes an assay for investigation of the maturation of transporter function, as well as, potentially, for analysis of more general aspects of transporter function. In this regard, there are two important advantages of these *in vitro* organ culture systems that bear mentioning. First, in each system there appears to be an open lumen in which substrate accumulates, suggesting that these systems may be used to study not just basolateral uptake, but the luminal efflux step of renal solute transport as well. Second, all aspects of gene expression are under endogenous promoter control and, thus, may more accurately reflect the *in vivo* pathways of gene regulation and expression and *in vivo* transporter protein levels.

Acknowledgments

The current affiliation for DHS is the Department of Pharmaceutical Sciences, Medical University of South Carolina, 280 Calhoun Street, Charleston, SC 29425 (address for reprint requests). This work was supported by an NICHHD grant, RO1-HD40011 (to SKN), NIDDK grants, R01-DK67216 (to DHS) and K08-DK64839 (to SAE) as well as a Normon S. Coplon Extramural Grant from Satellite Healthcare (to KTB). We gratefully acknowledge the invaluable technical assistance of Duke Vaughn. We also thank the UCSD QPCR facility and the VA Microscopy Core for technical assistance. The care and use of animals described in this investigation conform to the procedures of the laboratory's Animal Protocol approved by the Animal Subjects Program and the IACUC of the University of California, San Diego.

REFERENCES

1. Eraly SA, Monte JC, Nigam SK. Novel slc22 transporter homologs in fly, worm, and human clarify the phylogeny of organic anion and cation transporters. Physiol. Genomics 2004;18:12–24. [PubMed: 15054140]
2. Saier MH Jr. Beatty JT, Goffeau A, et al. The major facilitator superfamily. J Mol Microbiol Biotechnol 1999;1:257–279. [PubMed: 10943556]
3. Eraly SA, Bush KT, Sampogna RV, et al. The molecular pharmacology of organic anion transporters: From DNA to FDA? Mol Pharmacol 2004;65:479–487. [PubMed: 14978224]
4. Koepsell H. Polyspecific organic cation transporters: their functions and interactions with drugs. Trends in Pharmacological Sciences 2004;25:375–381. [PubMed: 15219980]
5. Wright SH, Dantzler WH. Molecular and Cellular Physiology of Renal Organic Cation and Anion Transport. Physiol. Rev 2004;84:987–1049. [PubMed: 15269342]
6. Buist SCN, Cherrington NJ, Choudhuri S, et al. Gender-Specific and Developmental Influences on the Expression of Rat Organic Anion Transporters. J Pharmacol Exp Ther 2002;301:145–151. [PubMed: 11907168]
7. Slitt AL, Cherrington NJ, Hartley DP, et al. Tissue Distribution and Renal Developmental Changes in Rat Organic Cation Transporter mRNA levels. Drug Metab Dispos 2002;30:212–219. [PubMed: 11792693]
8. Nakajima N, Sekine T, Cha SH, et al. Developmental changes in multispecific organic anion transporter 1 expression in the rat kidney. Kidney Int 2000;57:1608–1616. [PubMed: 10760096]
9. Pavlova A, Sakurai H, Leclercq B, et al. Developmentally regulated expression of organic ion transporters NKT (OAT1), OCT1, NLT (OAT2), and Roct. Am J Physiol Renal Physiol 2000;278:F635–643. [PubMed: 10751225]
10. Lopez-Nieto CE, You G, Barros EJG, et al. Molecular cloning and characterization of a novel transport protein with very high expression in the kidney (Abstract). J Am Soc Nephrol 1996;7:1301.
11. Lopez-Nieto CE, You G, Bush KT, et al. Molecular Cloning and Characterization of NKT, a Gene Product Related to the Organic Cation Transporter Family That Is Almost Exclusively Expressed in the Kidney. J. Biol. Chem 1997;272:6471–6478. [PubMed: 9045672]

12. Sweet DH, Wolff NA, Pritchard JB. Expression Cloning and Characterization of ROAT1. The Basolateral Organic Anion Transporter in Rat Kidney. J. Biol. Chem 1997;272:30088–30095. [PubMed: 9374486]
13. Sekine T, Watanabe N, Hosoyamada M, et al. Expression Cloning and Characterization of a Novel Multispecific Organic Anion Transporter. J. Biol. Chem 1997;272:18526–18529. [PubMed: 9228014]
14. Simonson G, Vincent A, Roberg K, et al. Molecular cloning and characterization of a novel liver-specific transport protein. J Cell Sci 1994;107:1065–1072. [PubMed: 8056831]
15. Sekine T, Cha SH, Tsuda M, et al. Identification of multispecific organic anion transporter 2 expressed predominantly in the liver. FEBS Lett 1998;429:179–182. [PubMed: 9650585]
16. Brady KP, Dushkin H, Fornzler D, et al. A novel putative transporter maps to the osteosclerosis (oc) mutation and is not expressed in the oc mutant mouse. Genomics 1999;56:254–261. [PubMed: 10087192]
17. Kusuhara H, Sekine T, Utsunomiya-Tate N, et al. Molecular Cloning and Characterization of a New Multispecific Organic Anion Transporter from Rat Brain. J. Biol. Chem 1999;274:13675–13680. [PubMed: 10224140]
18. Grundemann D, Gorboulev V, Gambaryan S, et al. Drug excretion mediated by a new prototype of polyspecific transporter. Nature 1994;372:549–552. [PubMed: 7990927]
19. Zhang L, Dresser MJ, Gray AT, et al. Cloning and Functional Expression of a Human Liver Organic Cation Transporter. Mol Pharmacol 1997;51:913–921. [PubMed: 9187257]
20. Okuda M, Saito H, Urakami Y, et al. cDNA cloning and functional expression of a novel rat kidney organic cation transporter, OCT2. Biochem Biophys Res Commun 1996;224:500–507. [PubMed: 8702418]
21. Gorboulev V, Ulzheimer JC, Akhoundova A, et al. Cloning and characterization of two human polyspecific organic cation transporters. DNA Cell Biol 1997;16:871–881. [PubMed: 9260930]
22. Tamai I, Yabuuchi H, Nezu J-i, et al. Cloning and characterization of a novel human pH-dependent organic cation transporter, OCTN1. FEBS Letters 1997;419:107–111. [PubMed: 9426230]
23. Wu X, Prasad PD, Leibach FH, et al. cDNA sequence, transport function, and genomic organization of human OCTN2, a new member of the organic cation transporter family. Biochem Biophys Res Commun 1998;246:589–595. [PubMed: 9618255]
24. Tamai I, Ohashi R, Nezu J-i, et al. Molecular and Functional Identification of Sodium Ion-dependent, High Affinity Human Carnitine Transporter OCTN2. J. Biol. Chem 1998;273:20378–20382. [PubMed: 9685390]
25. Schomig E, Spitzenberger F, Engelhardt M, et al. Molecular cloning and characterization of two novel transport proteins from rat kidney. FEBS Letters 1998;425:79–86. [PubMed: 9541011]
26. Cha SH, Sekine T, Kusuhara H, et al. Molecular Cloning and Characterization of Multispecific Organic Anion Transporter 4 Expressed in the Placenta. J. Biol. Chem 2000;275:4507–4512. [PubMed: 10660625]
27. Mori K, Ogawa Y, Ebihara K, et al. Kidney-specific expression of a novel mouse organic cation transporter-like protein. FEBS Letters 1997;417:371–374. [PubMed: 9409754]
28. Enomoto A, Kimura H, Chairoungdua A, et al. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. Nature 2002;417:447–452. [PubMed: 12024214]
29. Magagnin S, Werner A, Markovich D, et al. Expression cloning of human and rat renal cortex Na/Pi cotransport. Proc Natl Acad Sci U S A 1993;90:5979–5983. [PubMed: 8327470]
30. Lee WS, Kanai Y, Wells RG, et al. The high affinity Na+/glucose cotransporter. Reevaluation of function and distribution of expression. J Biol Chem 1994;269:12032–12039. [PubMed: 8163506]
31. Grobstein C. Mechanisms of organogenetic tissue interaction. Natl Cancer Inst Monogr 1967;26:279–299. [PubMed: 4864107]
32. Sariola H, Holm-Sainio K, Henke-Fahle S. The effect of neuronal cells on kidney differentiation. Int J Dev Biol 1989;33:149–155. [PubMed: 2485695]
33. Breen CM, Sykes DB, Fricker G, et al. Confocal imaging of organic anion transport in intact rat choroid plexus. Am J Physiol Renal Physiol 2002;282:F877–885. [PubMed: 11934698]

34. Sweet DH, Miller DS, Pritchard JB, et al. Impaired organic anion transport in kidney and choroid plexus of organic anion transporter 3 (Oat3 [Slc22a8]) knockout mice. J. Biol. Chem 2002;277:26934–26943. [PubMed: 12011098]
35. Sykes D, Sweet DH, Lowes S, et al. Organic anion transport in choroid plexus from wild-type and organic anion transporter 3 (Slc22a8)-null mice. Am J Physiol Renal Physiol 2004;286:F972–978. Epub 2003 Dec 2023. [PubMed: 15075193]
36. Sweet DH, Chan LM, Walden R, et al. Organic anion transporter 3 (Slc22a8) is a dicarboxylate exchanger indirectly coupled to the Na+ gradient. Am J Physiol Renal Physiol 2003;284:F763–769. [PubMed: 12488248]
37. Eraly SA, Hamilton BA, Nigam SK. Organic anion and cation transporters occur in pairs of similar and similarly expressed genes. Biochem Biophys Res Commun 2003;300:333–342. [PubMed: 12504088]
38. Urakami Y, Okuda M, Masuda S, et al. Functional Characteristics and Membrane Localization of Rat Multispecific Organic Cation Transporters, OCT1 and OCT2, Mediating Tubular Secretion of Cationic Drugs. J Pharmacol Exp Ther 1998;287:800–805. [PubMed: 9808712]
39. Karbach U, Kricke J, Meyer-Wentrup F, et al. Localization of organic cation transporters OCT1 and OCT2 in rat kidney. Am J Physiol Renal Physiol 2000;279:F679–687. [PubMed: 10997918]
40. Kato Y, Sai Y, Yoshida K, et al. PDZK1 Directly Regulates the Function of Organic Cation/Carnitine Transporter OCTN2. Mol Pharmacol 2005;67:734–743. [PubMed: 15523054]
41. Tamai I, China K, Sai Y, et al. Na(+)-coupled transport of L-carnitine via high-affinity carnitine transporter OCTN2 and its subcellular localization in kidney. Biochim Biophys Acta 2001;1512:273–284. [PubMed: 11406104]
42. Cheret C, Doyen A, Yaniv M, et al. Hepatocyte Nuclear Factor 1 [alpha] Controls Renal Expression of the Npt1-Npt4 Anionic Transporter Locus. Journal of Molecular Biology 2002;322:929–941. [PubMed: 12367519]
43. Martin MG, Wang J, Solorzano-Vargas RS, et al. Regulation of the human Na+-glucose cotransporter gene, SGLT1, by HNF-1 and Sp1. Am J Physiol Gastrointest Liver Physiol 2000;278:G591–603. [PubMed: 10762614]
44. Pontoglio M, Prie D, Cheret C, et al. HNF1{alpha} controls renal glucose reabsorption in mouse and man. EMBO Reports 2000;1:359–365. [PubMed: 11269503]
45. Pontoglio M, Barra J, Hadchouel M, et al. Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria, and renal Fanconi syndrome. Cell 1996;84:575–585. [PubMed: 8598044]

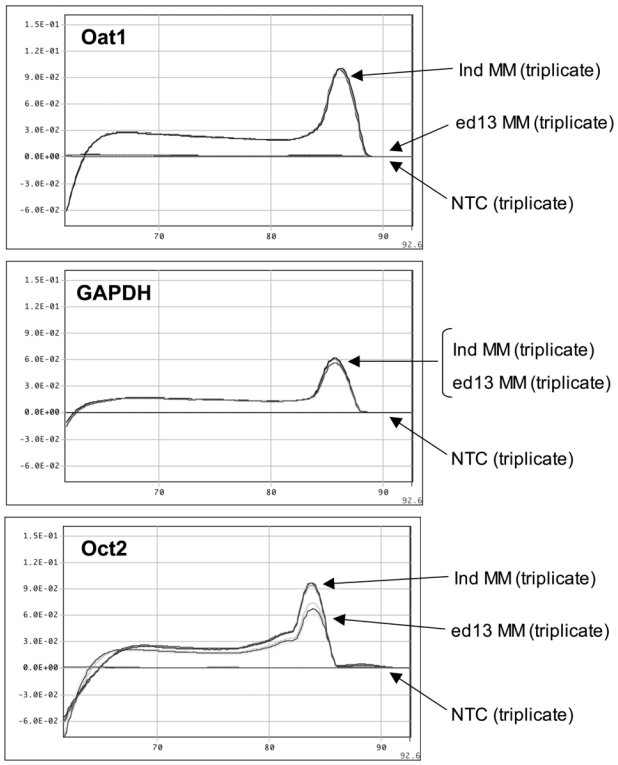

Figure 1. Representative QPCR dissociation curves

Dissociation curves for Oat1, Oct2, and GAPDH from one run of the induced MM analysis are shown and indicate the formation of a single specific PCR product for each primer pair. Dissociation curves for all primer pairs used in this study were similar to those shown. Also note that for Oat1, a gene for which the mRNA level was observed to change by 3-4 orders of magnitude after induction, there is a marked change in the PCR product peak areas. In contrast, the change in PCR product peak areas before and after induction for Oct2, a gene for which the mRNA level was observed to change by approximately 1 order of magnitude, is not as great.

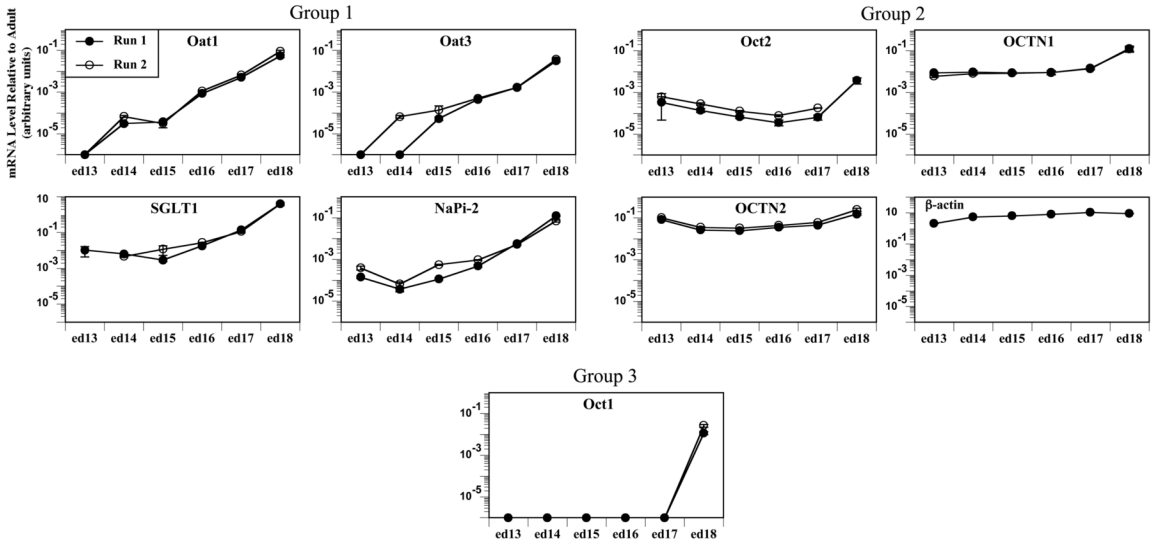

Figure 2. Relative gene expression levels of organic anion and cation transporter genes in embryonic kidney as determined by QPCR

RNA was prepared from embryonic kidneys at the indicated days of gestation, reverse-transcribed, and analyzed by QPCR (please refer to *Methods* for details). With the exception of β-actin, the expression of each gene at each time point was determined in samples prepared from two independent experiments (*i.e.*, run 1 and run 2) and each sample was analyzed in triplicate. Data were normalized to the level of expression of GAPDH in the same sample, and are reported as the ratio of the level of normalized gene expression in the sample of interest to the level of gene expression in adult kidney.

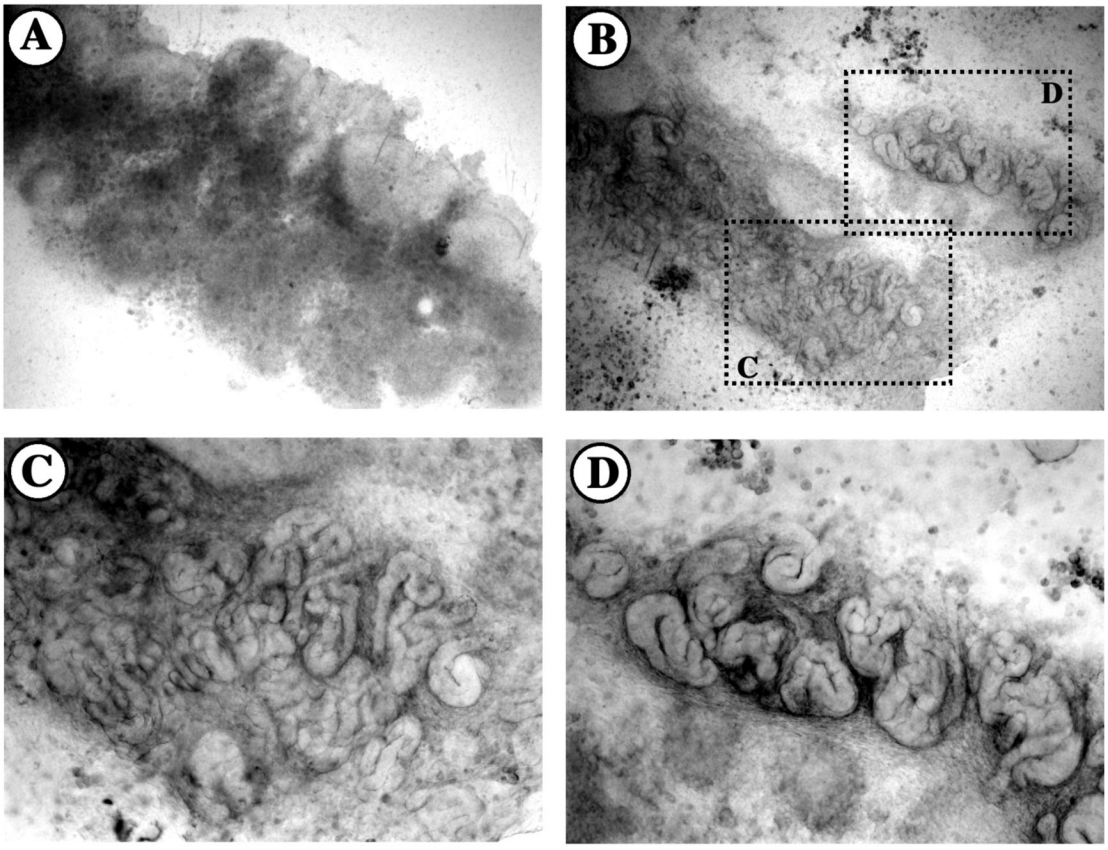

Figure 3. Transmitted light micrographs of induced metanephric mesenchyme

Twenty-four hours of co-culture of ed13 metanephric mesenchyme with ed13 spinal cord induces the subsequent formation of renal proximal tubule-like structures. Panel A: Rat ed13 MM tissue on Transwell filter membrane after 24 hr of co-culture (spinal cord tissue has been removed). Panel B: Induced proximal tubule-like structures 5 days after spinal cord-induction. Panels C and D: Higher magnification images of the regions indicated by dashed rectangles in panel B.

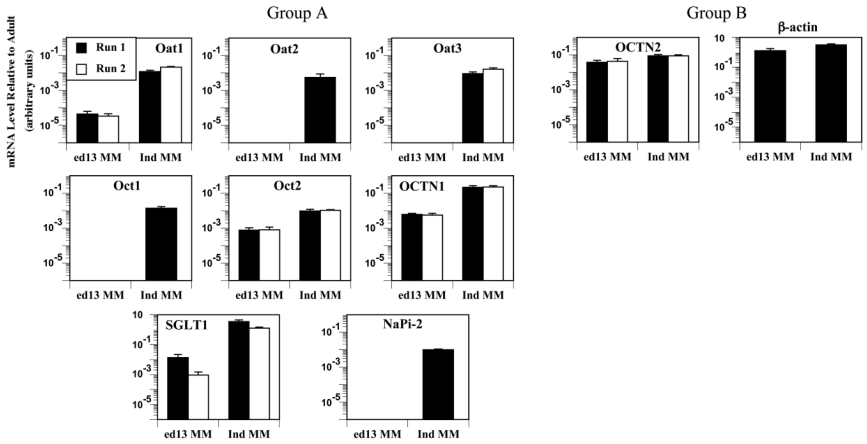

Figure 4. Relative gene expression levels of the indicated transporter genes in induced and un-induced metanephric mesenchyme as determined by QPCR

RNA was prepared from cultures of induced and un-induced metanephric mesenchyme (please refer to *Methods* for details), and QPCR analysis was performed as described in the legend for Fig. 2. Gene expression of the examined transporter genes could be divided into two general groups, those substantially upregulated by spinal cord induction (Group A) and those essentially unchanged between the induced and un-induced cultures (Group B).

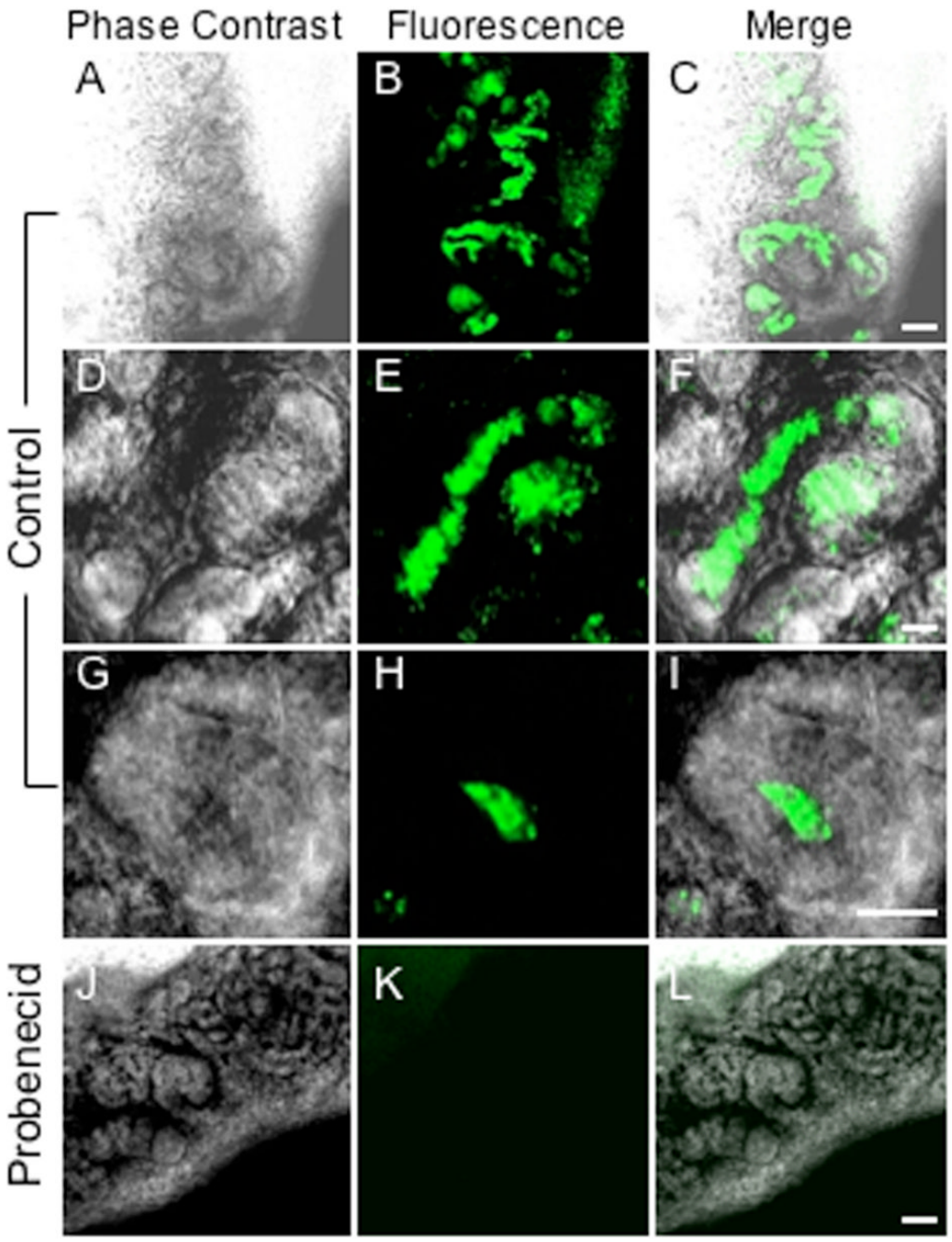

Figure 5. Confocal microscopic images showing fluorescein accumulation in induced metanephric mesenchyme

(A-L) Metanephric mesenchyme tissue isolated from ed13 rat kidneys was co-cultured with ed13 spinal cord tissue for 24 hr and then cultured for an additional 5 days following removal of the spinal cord tissue (please refer to *Methods* for details). Confocal microscopic images were acquired 30 minutes after exposure to 2 μM fluorescein in the culture medium in either the absence (Control; A-I) or presence (Probenecid; J-L) of 2 mM probenecid. (A, D, G, J) Phase contrast photomicrographs of the induced epithelial tubular structures. (B, E, H, K) Corresponding fluorescence photomicrograph of the same tissue shown in previous panel. (C, F, I, L) Merged image of the two previous photomicrographs indicating the accumulation of

fluorescein in the induced metanephric mesenchyme. (A-C) Low magnification examination of a group of tubular structures in the induced mesenchyme in the absence of probenecid; Bar = 100 μM. (D-F) High magnification examination of tubular structures in the induced mesenchyme in the absence of probenecid; Bar = 20 μM. (G-I) Higher magnification examination of a tubular structure in the induced mesenchyme in the absence of probenecid; Bar = 20 μM. Note the accumulation of fluorescein, to a concentration greater than the medium, in what appears to be a fluid filled space (presumptive lumen). (J-L) Low magnification examination of a group of tubular structures in the induced mesenchyme in the presence of 2 mM probenecid; Bar = 100 μM. Note the absence of concentrative fluorescein accumulation.

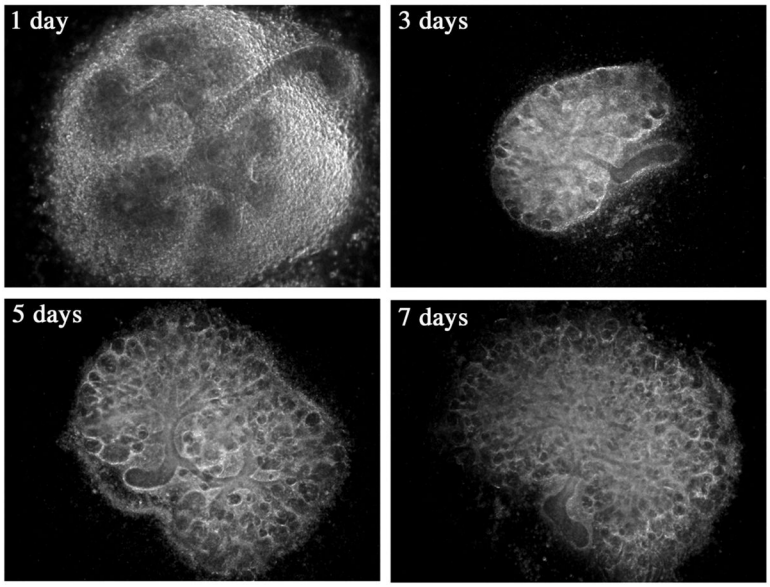

Figure 6. Dark-field images of whole embryonic kidney (WEK)

Culture of ed13 renal masses on transwell filter inserts for several days leads to the formation of small organ-like structures that resemble kidneys. The micrograph depicting WEK at one day of culture is at twice the magnification of the other micrographs.

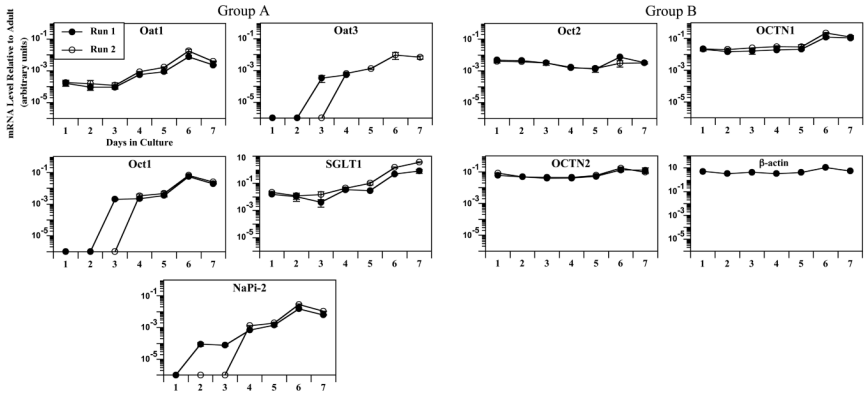

Figure 7. Relative gene expression levels of the indicated transporter genes in whole embryonic kidney culture as determined by QPCR

RNA was prepared from cultures of whole embryonic kidney (please refer to *Methods* for details) and QPCR analysis performed as described in the legend for Fig. 2, with the exception that each sample was analyzed in duplicate. Two major patterns of gene expression could be discriminated: Oat1, Oat3, Oct1, SGLT1, and NaP_i2 manifested a progressive increase in expression over the seven days of WEK culture (Group A), while Oct2, OCTN1, OCTN2, and β-actin expression did not substantially change over the duration of culture (Group B). Oat2, which was not detected in embryonic kidneys *in vivo*, was also undetectable in WEK culture.

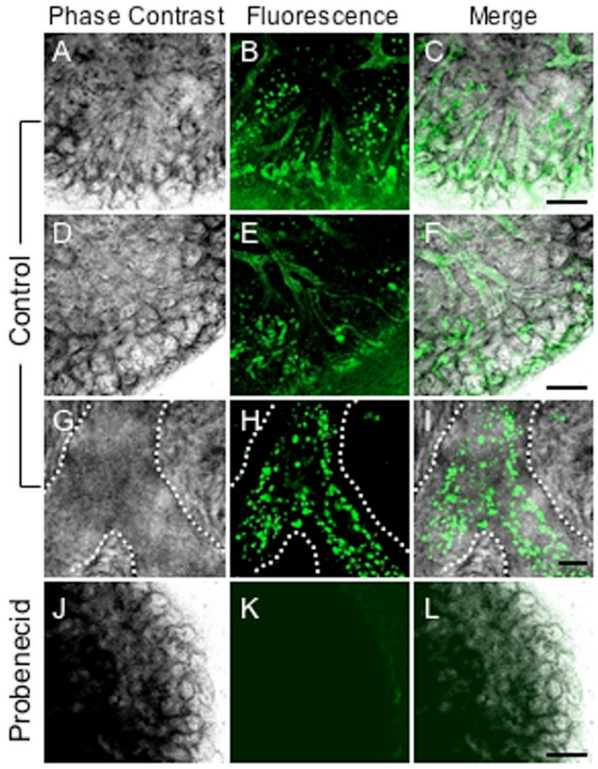

Figure 8. Confocal images showing fluorescein accumulation in cultured rat kidney

Embryonic day 13 kidneys isolated from rat embryos and maintained in organ culture for 7 days. Confocal images were acquired 30 minutes after exposure to 2 μM fluorescein in the culture medium in either the absence (Control; A-I) or presence (Probenecid; J-L) of 2 mM probenecid. (A, D, G, J) Phase contrast photomicrographs of the cultured kidneys showing the tissue structure. (B, E, H, K) Corresponding fluorescence photomicrograph of the same tissue shown in previous panel. (C, F, I, L) Merged image of the two previous photomicrographs indicating the accumulation of fluorescein in the cultured kidney. Note that the fluorescein concentration exceeds the medium concentration in specific compartments of the renal tissue. There is an intense fluorescent signal within underlying convoluted tubule-like structures (presumptive proximal tubules) near the surface of the organ, and a less intense signal present in larger, straighter branching structures (presumptive collecting ducts connected to the rest of the nephron) situated more deeply. (A-F) Low magnification examination of kidneys cultured in the absence of probenecid; Bar = 200 μM. (G-I) High magnification examination of collecting ducts found in kidneys cultured in the absence of probenecid. Basolateral surface of collecting duct is indicated by dashed white line; Bar = 20 μM. (J-L) Low magnification examination of a kidney cultured in the presence of 2 mM probenecid; Bar = 200 μM.

Table 1

Oligonucleotide primer pairs used for QPCR analysis.

Oat1for:	5′-ATGCCTATCCACACCCGTGC-3′
Oat1rev:	5′-GGCAAAGCTAGTGGCAAACC-3′
Oat2for:	5′-TAGCTCCTGCCTCCGATTTG-3′
Oat2rev:	5′-ACCACGGCTCCCACCAGCAC-3′
Oat3for:	5′-TGAGAAGTGTCTCCGCTTCG-3′
Oat3rev:	5′-CTGTAGCCAGCGCCACTGAG-3′
Oct1for:	5′-TGGCTGGGTATACGACACTC-3′
Oct1rev:	5′-TCCTGTAGCCAGAGCCGACA-3′
Oct2for:	5′-CTGGTGCATACCGGAATCTC-3′
Oct2rev:	5′-GAACAGAGCTCGTGAACCAA-3′
OCTN1for:	5′-ACTTCGAACCCTGCCTCGGAG-3′
OCTN1rev:	5′-CTTCTCTGTCCATGGAGACTG-3′
OCTN2for:	5′-CCATATCAGTGGGCTACTTCG-3′
OCTN2rev:	5′-CCCTGAGCATCTGGTCAATGG-3′
SGLT1for:	5′-TATACCTGGCCATTTTCATCC-3′
SGLT1rev:	5′-AAGGAGCTCATGAGGGAAGCC-3′
NaP*i*2for:	5′-CGACATCCATCATTGTCAGCA-3′
NaP*i*2rev:	5′-TTTCTCCATGGTGGTGTTTGC-3′
β-actinfor:	5′-CTTCCAGCCTTCCTTCCTGGGTATGG-3′
β-actinrev:	5′-GCGGACTGTTACTGAGCTGCGTTTTA-3′
GAPDHfor:	5′-TGCACCACCAACTGCTTAGC-3′
GAPDHrev:	5′-TGGATGCAGGGATGATGTTC-3′