Biochimica et Biophysica Acta, 1149 (1993) 145–150

BBAMEM 76015

Characterization of *p*-aminohippurate transport from rat kidney which is expressed after injection of size-selected mRNA into oocytes of *Xenopus laevis*

Jürgen Steffgen [a], Franz Scheyerl [a], Dirk Gründemann [b], Simone Kienle [a], Hans Eduard Franz [a] and Hermann Koepsell [b]

[a] *Zentrum für Innere Medizin, Universität Ulm, Ulm (Germany) and* [b] *Max-Planck-Institut für Biophysik, Frankfurt / Main, Germany*

(Received 27 January 1993)

Key words: Oocyte; Expression; Organic anion transporter; *p*-Aminohippuric acid; Probenecid; (*X. laevis*)

First, the existence of an endogeneous *p*-aminohippurate (PAH) transporter in oocytes of *Xenopus laevis* was demonstrated. When, however, the oocytes were injected with mRNA from rat kidney cortex, an expressed *p*-aminohippuric acid (PAH) uptake was seen which differed from the endogeneous transporter. Both transport systems are saturated at high PAH concentrations, exhibit trans-stimulation by PAH and are partially inhibited by probenecid. The endogeneous transport has a rather low affinity for PAH (K_m = 0.57 mM) and is about 50% inhibited by probenecid (one apparent inhibition site with half maximal inhibition at 0.5 mM). The expressed PAH transport has a high affinity for PAH (K_m = 60 μM) and can be inhibited 80% by probenecid (two apparent inhibition sites with half maximal inhibitions at 1 μM and 2 mM). Expression experiments with fractionated mRNA revealed that the PAH transport expressed from rat kidney cortex is encoded by an mRNA of 1.8 to 2.5 kb.

Introduction

Most vertebrates are capable of secreting *p*-aminohippurate (PAH) and other anions (e.g., sulfate- or glucoronate-conjugated drugs) by the proximal renal tubules into the urine [1–5]. This process involves uptake against an electrochemical gradient into the epithelial cells at the basolateral membrane and outward movement of the ions at the luminal membrane. PAH-anion exchangers on both membrane surfaces have been characterized in different species, however, the question whether the transporters on both membrane surfaces are different has not been conclusively answered since large species differences exist in PAH transport [6] and the transport exhibits an astonishing poor substrate specificity [7–9].

Since attempts to identify PAH transporters by affinity labeling and/or purification experiments have failed, expression cloning in oocytes of *Xenopus laevis* was considered a promising method to elucidate the molecular structure of PAH transporters. Expression of PAH uptake in *Xenopus* oocytes by renal mRNA has been reported earlier, however, the expressed uptake was characterized rather incompletely and the expression of PAH uptake was observed in mRNA fractions of different size [10,11]. After injection of mRNA from rabbit kidney expression of saturable, low affinity PAH uptake was observed which was not inhibited by 1 mM probenecid [10], whereas probenecid-inhibitable PAH uptake of non-specified affinity was detected after injection of mRNA from rat kidney [11]. Thus, to start with expression cloning experiments a more detailed characterization of the expressed PAH uptake in *Xenopus* oocytes appeared to be necessary.

In *Xenopus* oocytes we observed endogeneous saturable PAH uptake which was inhibited by probenecid, and were able to express additional PAH uptake by injection of rat renal mRNA. Distinct differences between the endogeneous and the expressed PAH transport were detected and a functional assay for expression cloning of PAH transporters established. Part of the results has been reported as abstract [12].

Materials and Methods

Materials

p-[*glycyl*-2-^{3}H]Aminohippuric acid (165 GBq/mmol) was supplied by Du Pont de Nemours (Dreieich, Ger-

Correspondence to: H. Koepsell, Max-Planck-Institut für Biophysik, Kennedyallee 70, 60596 Frankfurt 70, Germany.

Abbreviations: PAH, *p*-aminohippurate; DEPC, diethyl pyrocarbonate.

many), streptomycin sulfate by Serva (Heidelberg, Germany) and gentamycin sulfate by Fluka Chemie (Buchs, Switzerland). Collagenase type IA from *Clostridium histolyticum* (1.3 units/mg), diethyl pyrocarbonate (DEPC) and RNA size markers were obtained from Sigma (München, Germany). All other chemicals were obtained as described earlier [13].

Purification, fractionation and detection of mRNA

Total RNA was isolated from rat kidney cortex [14] and mRNA was purified by affinity chomatography on oligo(dT) cellulose [15], precipitated with sodium acetate/ethanol, washed with ethanol, vacuum-dried, suspended in H_2O and frozen in liquid nitrogen [16]. The mRNA concentration was determined spectrophotometrically at 260 nm. For fractionation 175 μg of mRNA was applied on top of a non-denaturating 1.2% (w/v) agarose gel as described by Sambrook et al. [16]. After electrophoresis vertical strips were stained with ethidium bromide and the remaining gel was cut into horizontal strips from which mRNA was eluted electrically, precipitated with sodium acetate/ethanol, washed with ethanol and resuspended in water. The electroelution was performed by a procedure which has been described for the elution of DNA [16]. For the analysis of mRNA also denaturating agarose gels in the presence of formaldehyde and formamide were performed [17].

Expression of PAH transport in oocytes

From anesthetized female *Xenopus laevis* clawed toads ovary sections were removed, dissociated mechanically and incubated overnight (18°C) in 5 mM Hepes-Tris (pH 7.4), 90 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ (ORi) containing 3 mg/ml of collagenase, 20 mg/l penicillin and 25 mg/ml streptomycin. Oocytes arrested in the prophase of the first meiotic division (type V or VI after Dumont [18]) were selected, incubated 10 min in Ca^{2+}-free ORi (22°C) and stored in ORi (18°C). The oocytes were injected with 50 nl of DEPC-treated water with or without (controls) different amounts of mRNA. For expression of transport the injected oocytes were incubated for 3 days in ORi containing 50 mg/l gentamycin (18°C, buffer exchange after 24 and 48 h).

Transport measurements

Endogeneous and expressed PAH uptake was measured in the presence of Na^+ (ORi) and in the absence of Na^+ (5 mM Hepes-Tris (pH 7.4), 93 mM tetramethylammonium chloride, 2 mM $CaCl_2$, 1 mM $MgCl_2$ (sodium-free ORi)). The measurements were performed in non-preloaded oocytes and in oocytes which were preloaded with PAH by incubating them for 15 h (18°C) in ORi containing 10 mM PAH. Preloaded oocytes and those which were employed for measurements performed in the absence of Na^+ were washed 4-fold (0°C) with sodium-free ORi. For transport measurements the oocytes were incubated (21°C) for different time intervals with [^{3}H]PAH dissolved in ORi or in sodium-free ORi. Nonspecific uptake was determined by performing the measurements in the presence of 10 mM probenecid or of 5 mM non-labeled PAH. After the incubation with [^{3}H]PAH the oocytes were washed 4fold with ice cold ORi, solubilized with 5% (w/v) SDS and analyzed for radioactivity by liquid scintillation counting. The presented data represent means of 6–10 determinations in different oocytes ± S.E. (S.D./n). To determine the K_m values for endogeneous and expressed PAH transport the Michaelis-Menten equation was fitted to the data in Fig. 3.

Results

Detection of endogeneous and expressed probenecid-inhibitable PAH uptake in Xenopus oocytes

When oocytes were incubated with 2 μM [^{3}H]PAH (30 min, 22°C) in the presence of Na^+, [^{3}H]PAH uptake was obtained which could be partially inhibited by probenecid. In oocytes from one animal similar endogeneous uptake rates were measured (see the S.E. values indicated below, each of which was calculated from 6–10 oocytes of one female). However, in oocytes from different animals, the PAH uptake rates and the degrees of inhibition by 10 mM probenecid varied considerably. Thus, in different animals the endogeneous probenecid-inhibitable uptake of 2 μM [^{3}H]PAH ranged from 5.6 ± 2.2 to 216 ± 5 fmol/oocyte per h in our experiments (mean value ± S.E. of 16 different batches of oocytes: 70 ± 15 fmol/oocyte per h). After addition of 10 mM probenecid the PAH uptake was inhibited between 13 and 86% (mean value ± S.E. of the pooled data, 42 ± 6%). When we injected 50 ng of poly(A)$^+$ mRNA which was isolated from rat kidneys, the uptake rates of 2 μM [^{3}H]PAH were stimulated between 1.4 and 17-fold over those measured in non-injected or in water-injected oocytes (experiments with 14 different batches of oocytes in which mRNA from five different preparations was injected). The expressed PAH uptake was inhibited between 70 and 95% (mean value ± S.E. of the pooled data, 82 ± 9%) by 10 mM probenecid. The expressed probenecid-inhibitable PAH uptake per oocyte ranged from 25 ± 6 to 1141 ± 58 fmol/oocyte per h (mean value ± S.E. of the pooled data, 369 ± 70 fmol/h).

In Fig. 1a the time-course of probenecid-inhibitable [^{3}H]PAH uptake is shown. Since endogeneous and expressed uptake was linear for about 30 min this incubation period was employed for uptake measurements with unloaded oocytes performed in the presence of Na^+. Fig. 1b shows the expression of probenecid-inhibitable [^{3}H]PAH uptake after injection

of increasing amounts of mRNA. Maximal expression was observed when about 50 ng of mRNA were injected per oocyte.

Substrate-dependence and probenecid inhibition of endogeneous and expressed PAH uptake

To test for functional differences between the endogeneous and the expressed PAH transport the dose-dependence of transport inhibition by probenecid was investigated and the substrate dependence of probenecid-inhibitable PAH uptake was measured. Distinct differences between endogeneous and expressed PAH uptake were detected. Fig. 2 shows that the probenecid-sensitivity of endogeneous and expressed PAH uptake was different. Thus, for the endogeneous uptake one apparent inhibition site with half-maximal inhibition at about 500 μM was detected and on the average (see above) about one half of the endogeneous PAH uptake could be inhibited. For the expressed uptake two apparent probenecid inhibition sites were found. A high affinity site with half-maximal inhibition at about 1 μM by which in most preparations (see above) about 50% of the expressed uptake was inhibited and a low-affinity site with half-maximal inhibition at about 2 mM by which on the average another 40% of the expressed uptake could be inhibited. In Fig. 3 the substrate dependence of PAH uptake in the presence of Na^+ which could be inhibited by 10 mM probenecid was evaluated. For endogeneous and expressed PAH uptake K_m values of 0.57 ± 0.10 mM and 0.060 ± 0.07 mM were obtained (Fig. 3). The data indicate different probenecid sensitivity and substrate dependence of the endogeneous and expressed probenecid-inhibitable PAH uptake.

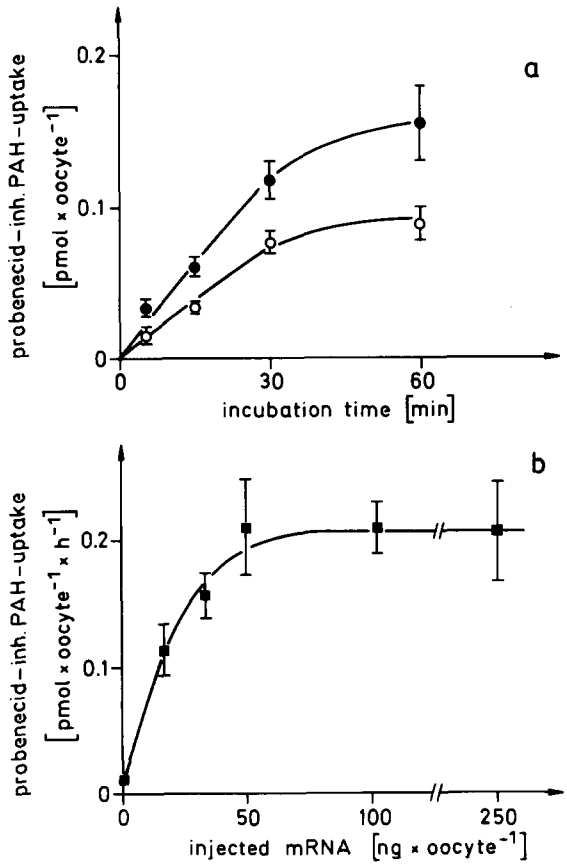

Fig. 1. Time-course of endogeneous and expressed, probenecid-inhibitable PAH uptake (a) and mRNA-dependence of expressed, probenecid-inhibitable PAH uptake (b) measured in the presence of Na^+. Oocytes of *Xenopus laevis* were injected with water or with different amounts of poly(A)$^+$ mRNA from rat kidney and incubated for 3 days at 18°C. For transport measurements the oocytes were incubated in ORi buffer containing 2 μM [^{3}H]PAH with and without 10 mM probenecid, and the amount of [^{3}H]PAH in the oocytes was measured. The measurements were performed after different time intervals of incubation (a) or after 30 min of incubation (b) and the probenecid-inhibitable PAH uptake was calculated by subtracting the nonspecific uptake from the total uptake. Panel a demonstrates the endogeneous (○) and the expressed (●) probenecid-inhibitable PAH uptake after different times of incubation. Panel b shows the probenecid-inhibitable PAH uptake rate expressed by the indicated amounts of injected mRNA.

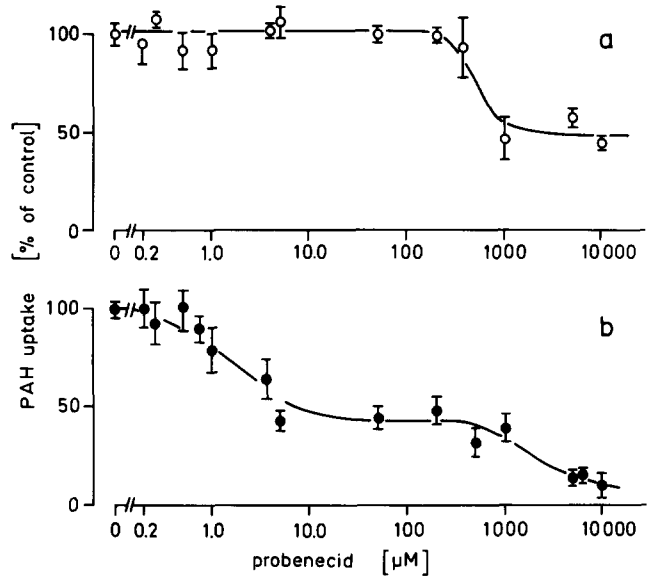

Fig. 2. Dose-response curve of probenecid inhibition of endogeneous (○) and expressed (●) PAH transport measured in the presence of Na^+. Oocytes were injected and incubated as in Fig. 1. Then the uptake of 2 μM PAH was measured after 30 min incubation in the presence of Na^+ and the indicated concentrations of probenecid. Panel a shows the endogeneous uptake rates calculated as percentage of the uptake measured in the absence of probenecid. Panel b shows the expressed uptake rates which were calculated by subtracting the endogeneous uptake rates measured in the presence of the indicated probenecid concentrations from the uptake rates in the mRNA-injected oocytes measured in the presence of the respective probenecid concentrations.

Trans-stimulation and Na^+-dependence of PAH uptake

It was observed that the endogeneous and the expressed probenecid-inhibitable PAH uptake measured in the presence of Na^+ was increased when the oocytes were preloaded with PAH (preloading was performed

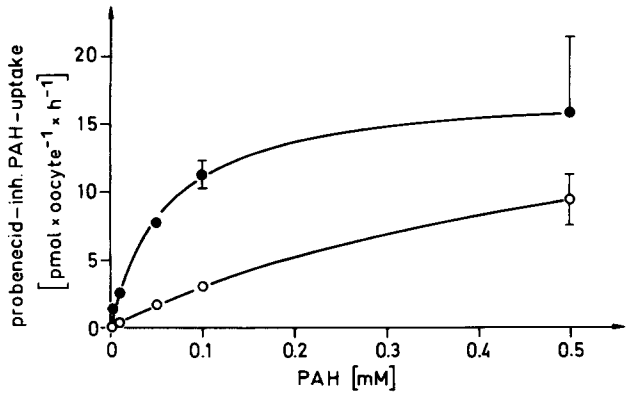

Fig. 3. Substrate dependence of endogeneous (○) and expressed (●) probenecid-inhibitable PAH uptake measured in the presence of Na^+. Oocytes of *Xenopus laevis* were injected with water or with 50 ng of mRNA per oocyte and incubated for 3 days. The PAH uptake was measured after 30 min incubation in the presence of Na^+ and different concentrations of PAH. The open symbols show the probenecid-inhibitable fraction of the endogeneous uptake and the closed symbols the probenecid-inhibitable uptake obtained after mRNA injection minus the probenecid-inhibitable endogeneous uptake. The data were fitted as described in Materials and Methods.

as described in Materials and Methods. In control experiments employing [^{3}H]PAH the intracellular PAH concentration after the preincubation was determined to be about 2 mM). This indicates trans-stimulation by PAH (data not shown). When during the transport measurements Na^+ was replaced by tetramethylammonium, endogeneous and expressed PAH uptake (probenecid-inhibitable and saturable) were reduced by varying degrees in different animals. Table I shows five experiments with different batches of oocytes. In the absence of Na^+, mRNA-expressed probenecid-inhibitable and saturable PAH uptake was only observed in about 50% of the experiments. In six experiments with expression of PAH transport, uptake of 2 μM PAH measured in the absence of Na^+ after preloading with PAH was stimulated by a factor of 2.8 ± 0.3 when 10 ng of mRNA were injected per oocyte. In Fig. 4 PAH transport measurements are demonstrated which were performed in the absence of Na^+. They are typical for the oocyte batches in which PAH transport was observed in the absence of Na^+. The oocytes in Fig. 4 were injected with water or with 10 ng/oocyte of total mRNA, and uptake of 2 μM PAH was measured in the absence of Na^+ with and without preloading of the oocytes with PAH. Fig. 4 shows that the endogenous and the expressed PAH uptake can be measured in the absence of Na^+ and are significantly stimulated by preloading the oocytes with PAH.

Size-fractionation of the mRNA encoding for probenecid-inhibitable PAH-anion exchange

Poly(A)$^+$ mRNA was size-fractionated by agarose gel electrophoresis (Fig. 5). The mRNA fractions were eluted electrically and their concentration was determined. 10 ng mRNA per oocyte were injected and the probenecid-inhibitable uptake of 2 μM PAH was measured in the presence of Na^+ (Table II). After injec-

TABLE I

Effect of sodium removal on endogeneous and expressed probenecid-inhibitable PAH uptake

Oocytes from five different animals were injected with water or with 50 ng of poly(A)$^+$ mRNA from rat kidney. After three days incubation the uptake of 2 μM PAH was measured in the presence and absence of Na^+, with and without 10 mM probenecid present. The fractions of PAH uptake inhibited by probenecid were calculated. The expressed uptake rates were determined by subtracting the endogeneous PAH uptake from the uptake measured after injection of mRNA.

Animal	Sodium	Probenecid-inhibitable PAH uptake (fmol/oocyte per h)	
		endogeneous	expressed
1	+	99±27	279±42
	−	64±13	59±20
2	+	35± 5	628±40
	−	32± 8	3± 9
3	+	83± 9	187±20
	−	64± 9	7±14
4	+	5± 4	103±22
	−	1± 3	88±13
5	+	79±26	321±39
	−	69± 8	232±32

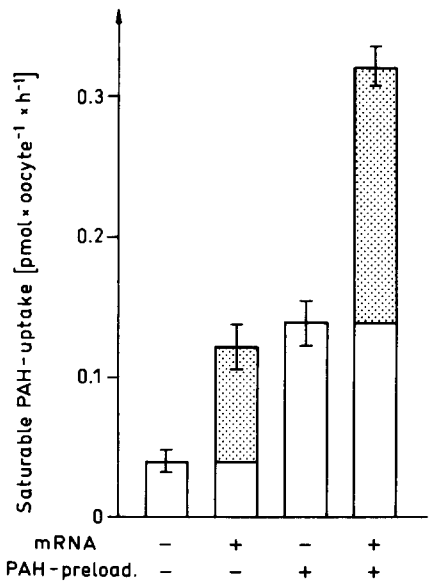

Fig. 4. Measurements of endogeneous and expressed PAH uptake in the absence of Na^+. Oocytes were injected with water or with mRNA (10 ng per oocyte) as indicated. After 3 days incubation part of the oocytes were preloaded with PAH and all oocytes were washed in Na^+-free buffer. The uptake of 2 μM [^{3}H]PAH was measured after 5 min incubation in Na^+-free buffer and corrected for [^{3}H]PAH uptake measured in the presence of 5 mM non-labeled PAH. The fraction of uptake which was increased over the endogeneous uptake by the mRNA injection is indicated by hatched bars.

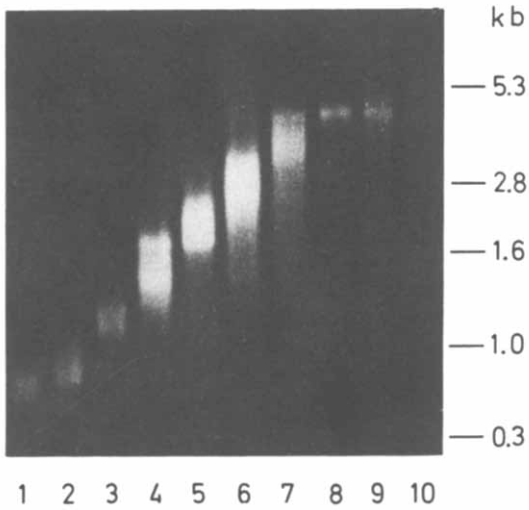

Fig. 5. Agarose gel electrophoresis of size-fractionated and electroeluted mRNA samples. Poly(A)$^+$ mRNA from rat kidney was fractionated on a non-denaturating agarose gel and 10 mRNA-fractions were electro-eluted from horizontal gel-strips. The figure shows an ethidium bromide-stained denaturating agarose gel onto which aliquots of the indicated electro-eluted mRNA fractions 1–10 were applied. Size standards for mRNA which were run on the same gel are indicated on the right.

tion of mRNA (10 ng) from fraction 5 the PAH uptake was about 5-fold higher than after injection of poly(A)$^+$ mRNA (Table II). A similar result was obtained with a different batch of oocytes by measuring the uptake of 2 μM PAH in the absence of Na^+ after preloading of the oocytes with PAH (see Materials and Methods). In this experiment 4-fold higher expression was observed with mRNA fraction 5 than with total mRNA. The data indicate a partial purification of the mRNA encoding for the probenecid-inhibitable PAH/anion exchanger from rat kidney. The encoding mRNA is supposed to contain between 1.8 and 2.5 kilobases.

TABLE II

Expression of PAH uptake in size-fractionated poly(A)$^+$ mRNA from rat kidney

Water, non-fractionated poly(A)$^+$ mRNA (10 ng/oocyte) and fractions (single and pooled) of size-fractionated poly(A)$^+$ mRNA (10 ng/oocyte, same experiment and numbering as in Fig. 5) were injected into oocytes. After three days incubation the uptake of 2 μM PAH was measured after 30 min incubation in the presence of Na^+ with and without 10 mM probenecid. The fractions of uptake inhibited by probenecid were calculated and the uptake rates in water-injected oocytes were subtracted from those obtained after mRNA injection.

mRNA	Expressed probenecid-inhibitable PAH uptake (fmol/oocyte per h)
poly(A$^+$) mRNA	149 ± 52
fract. 1–3	9 ± 18
fract. 4	3 ± 6
fract. 5	791 ± 57
fract. 6	475 ± 34
fract. 7	66 ± 25
fract. 8–10	120 ± 22

Discussion

The present investigation shows that oocytes of *Xenopus laevis* possess an endogeneous PAH transporter which can be inhibited by probenecid and that additional PAH transport activity can be expressed by injection of mRNA from rat kidney cortex. The endogeneous and expressed PAH transporters differ in respect to the affinity for PAH and to the sensitivity for probenecid. In the endogeneous uptake the apparent K_m for PAH is one order of magnitude higher than in the mRNA expressed uptake. One endogeneous low-affinity inhibition site of probenecid (half-maximal inhibition at about 500 μM) was detected whereas two apparent inhibition sites (half maximal inhibitions at about 1 μM and 2 mM) were observed in the expressed uptake. The endogeneous and the expressed PAH transport activity was demonstrated in the absence and presence of sodium and was trans-stimulated by PAH. Although the K_m value of the expressed PAH-uptake agrees reasonably with the K_m value of 0.08 mM which was determined for the basolateral PAH uptake in rat kidney proximal tubules [19], we do not know whether the PAH-anion exchanger from the basolateral or from the luminal membrane has been expressed, since both transporters may be identical. Thus, recent PAH transport measurements in membrane vesicles from bovine renal proximal tubules revealed similar K_m values and substrate specificities in luminal and basolateral membranes [20]. Since after size-fractionation of mRNA PAH transport was mainly expressed in a subfraction containing mRNA between 1.8 and 2.5 kb, the molecular masses of the encoded protein(s) are estimated to be between 50 and 90 kDa. This is consistent with the 74 kDa which has been determined for probenecid-inhibitable PAH-uptake in rabbit kidney cortex by radiation inactivation [21]. Since the oocytes contain an endogeneous PA transporter it cannot be decided whether we have expressed a PAH-transporting protein or a protein which modifies the kinetics, activity and/or expression of the endogeneous PAH transporter [22,23]. We also do not know whether the expressed PAH uptake is mediated by one PAH transporter with two probenecid inhibition sites or by two different PAH transporters.

After the functional differences between the endogeneous PAH transport in *Xenopus* oocytes and the PAH transport expressed from rat kidney mRNA have been established, it is possible to choose experimental conditions which primarily detect the expressed uptake. Thus, high-affinity uptake of a micromolar PAH-concentration should be measured and corrected for

nonspecific plus endogeneous PAH uptake by subtracting uptake measurements which are performed either in the presence of 200 μM nonlabeled PAH or in the presence of 100 μM probenecid. We recommend performing the uptake measurements in the presence of Na^+, since the oocytes are more stable and the expression of PAH uptake is observed more consistently in different batches of oocytes in the presence of Na^+. Since also with Na^+ considerable variations were observed in different batches of oocytes, in each round of screening during expression cloning the quality of the employed oocyte batch should be tested with an active sample of renal mRNA.

The large variability of PAH uptake measured in the absence of Na^+ which was observed in oocytes from different females, is not understood. One reason may be the variability of the membrane potential in the absence of Na^+. Vasilets and co-workers showed that the resting potential in *Xenopus* oocytes decreased from -60 mV to about -100 mV when Na^+ was removed from the bath [24]. Na^+ removal leads to an activation of the endogeneous Na^+/K^+-ATPase which contributes significantly to the membrane potential under these conditions. The well-known variation of endogeneous Na^+/K^+-ATPase activity in oocytes from different females is supposed to be the reason for the varying membrane potential. The membrane potential will influence the rate of PAH uptake if PAH transport has a potential-sensitive step in the overall electroneutral transport cycle. Another explanation for the observed apparent Na^+-dependence of PAH transport is that Krebs-cycle intermediates which leak out from the oocytes, may be transported back into the oocytes via endogeneous or expressed Na^+-dicarboxylate cotransporters and may, thus, stimulate PAH uptake via the Na^+-independent PAH transporter [25–27].

Ackowledgements

This work was supported by Deutsche Forschungsgemeinschaft (SFB169). The authors thank Prof. Dr. K.J. Ullrich for continuous support and stimulating discussions.

References

1 Weiner, I.M. (1973) in Handbook of Physiology, Section 8: Renal Physiology (Orloff, J. and Berliner, R.W., eds.), pp. 521–554, Oxford University Press, Oxford.
2 Häberle, D.A. (1981) in Renal Transport of Organic Substances (Greger, R., Lang, F., Silbernagl, S., eds.), pp. 189–209, Springer, Berlin.
3 Møller, J.V. and Sheikh, M.I. (1983) Pharmacol. Rev. 34, 315–358.
4 Pritchard, J.B. (1987) Biochim. Biophys. Acta 906, 295–308.
5 Roch-Ramel, F., Besseghir, K. and Murer, H. (1992) in Handbook of Physiology, Vol. II, Section 8: Renal Physiology (Windhager, E.E., ed.), pp. 2189–2262, Oxford University Press, Oxford.
6 Lang, F. (1981) in Renal Transport of Organic Substances (Greger, R., Lang, F. and Silbernagl, S., eds.), pp. 234–261, Springer, Berlin.
7 Ullrich, K.J. and Rumrich, G. (1988) Am. J. Physiol. 254, F453–F462.
8 Ullrich, K.J. and Rumrich, G. (1992) Pflügers Arch. 421, 286–288.
9 Ullrich, K.J., Rumrich, G., Fritzsch, G. and Klöss, S. (1987) Pflügers Arch. 408, 38–45.
10 Kwon, O., Kwon, H.M., Hong, S.K. and Goldinger, J.M. (1989) Proc. Soc. Exp. Biol. Med. 192, 205–208.
11 Wolff, N.A., Philpot, R.M., Miller, D.S. and Pritchard, J.B. (1992) Mol. Cell. Biochem. 114, 35–41.
12 Steffgen, J., Schwarz, W. and Koepsell, H. (1990) Pflügers Arch. 415, S1, R28.
13 Sander, I., Lottspeich, F., Appelhans, H., Kojro, E., Spangenberg, J., Weindel, C., Haase, W. and Koepsell, H. (1992) Biochim. Biophys. Acta 1112, 129–141.
14 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. 162, 156–159.
15 Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408–1412.
16 Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.
17 Kroczek, R. and Siebert, E. (1990) Anal. Biochem. 184, 90–95.
18 Dumont, J.N. (1972) J. Morphol. 136, 153–180.
19 Ullrich, K.J., Rumrich, G., Fritzsch, G. and Klöss, S. (1987) Pflügers Arch. 409, 229–235.
20 Schmitt, C. and Burckhardt, G. (1993) Pflügers Arch. 423, 280–290.
21 Kwon, H.M. (1990) Biochim. Biophys. Acta 1027, 253–256.
22 Veyhl, M., Püschel, B., Spangenberg, J., Dekel, C. and Koepsell, H. (1992) FASEB J. 6, A1459.
23 Bertran, J., Werner, A., Moore, M.L., Stange, G., Markovich, D., Biber, J., Testar, X., Zorzano, A., Palacin, M. and Murer, H. (1992) Proc. Natl. Acad. Sci. USA 89, 5601–5605.
24 Vasilets, L.A., Ohta, T., Noguchi, S., Kawamura, M. and Schwarz, W. (1993) Eur. Biophys. J. 21, 433–443.
25 Shimada, H., Moewes, B. and Burckhardt, G. (1987) Am. J. Physiol. 253, F795–F801.
26 Chatsudthipong, V. and Dantzler, W.H. (1991) Am. J. Physiol. 261, F858–F867.
27 Pritchard, J.B. (1988) Am. J. Physiol. 255, F597–F604.