Expression of the Hepatocyte Na+/Bile Acid Cotransporter in Xenopus laevis Oocytes*

(Received for publication, October 6, 1989)
Bruno Hagenbuch‡, Herman Lübbert§, Bruno Stieger†, and Peter J. Meier††
From the ‡Division of Clinical Pharmacology, Department of Medicine, University Hospital, CH-8091 Zurich, Switzerland, and the §Preclinical Research Department, Sandoz Ltd., CH-4002 Basel, Switzerland.

The expression of the basolateral Na+/bile acid (taurocholate) cotransport system of rat hepatocytes has been studied in Xenopus laevis oocytes. Injection of rat liver poly(A)+ RNA into the oocytes resulted in the functional expression of Na+ gradient stimulated taurocholate uptake within 3–5 days. This Na+-dependent portion of taurocholate uptake exhibited saturation kinetics (apparent Kₐ ≈ 91 μM) and could be inhibited by 4,4'-diisothiocyanato-2,2'-disulfonic acid stilbene. Furthermore, the expressed taurocholate transport activity demonstrated similar substrate inhibition and stimulation by low concentrations of bovine serum albumin as the basolateral Na+/bile acid cotransport system previously characterized in intact liver, isolated hepatocytes, and isolated plasma membrane vesicles. Finally, a 1.5–3.0-kilobase-size-class of mRNA could be identified that was sufficient to express the basolateral Na+/taurocholate uptake system in oocytes. These results demonstrate that “expression cloning” represents a promising approach to ultimately clone the gene and to further characterize the molecular properties of this important hepatocellular membrane transport system.

Continuous vectorial secretion of bile acids from blood into bile is an essential function of hepatocytes in all vertebrate animal species. In mammals the first step in this overall transcellular transport process is mediated by a secondary active, Na+ gradient-driven uptake system at the basolateral (sinusoidal and lateral) surface domain of hepatocytes. This Na+/bile acid cotransport system has been well characterized in a number of experimental systems (e.g. perfused rat liver, isolated hepatocytes, plasma membrane vesicles) with respect to its driving force, its transport kinetics, and its broad substrate specificity (1–4). Furthermore, the involved transport protein has been identified as a membrane polypeptide with an apparent molecular weight of 49,000 based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis (5). However, the exact molecular structure of the hepatocellular Na+/bile acid cotransporter has not yet been determined nor has the transport system been characterized on the mRNA and cDNA levels. Indeed, it might be difficult to achieve this goal solely through isolation, reconstitution, and partial sequencing of the protein, since the basolateral membrane contains a variety of closely related transporting polypeptides in the 48–54 kDa region (1, 2, 5–7). Therefore, we have attempted to functionally express the hepatocellular Na+/bile acid cotransporter in Xenopus laevis oocytes and to identify the mRNA size-class that encodes the carrier protein. This may serve as the first step in an “expression cloning” strategy, which is independent of probes such as antibodies and synthetic oligonucleotides and has been successfully used in recent years to characterize and clone several important membrane proteins including the Na+/glucose cotransporter of rabbit and human small intestine (8–10), ion channels (11, 12), and neurotransmitter-receptors (13–15). The present study demonstrates that injection of rat liver poly(A)+ RNA into X. laevis oocytes resulted in the functional expression of the hepatocellular Na+/bile acid cotransporter within 3–5 days. Furthermore, a 1.5–3.0-kilobase-size-class of mRNA was sufficient to generate the Na+-dependent bile acid (taurocholate) uptake signal. Hence, expression cloning represents a promising approach to ultimately clone the gene of the basolateral Na+/bile acid cotransport system of rat liver.

EXPERIMENTAL PROCEDURES

Materials—[6-3H]Taurocholic acid (2.1–6.6 Ci/mmol) was obtained from Du Pont-New England Nuclear. Guanidinium isothiocyanate, phenol, and sucrose were from Bethesda Research Laboratories. Oligo(dT)-cellulose (Type 7) was obtained from Pharmacia LKB Biotechnology Inc. Ethyl m-aminobenzoate (MS-222) was purchased from Sandoz Ltd., Basel, Switzerland.

Animals—Male Sprague-Dawley rats (SUT/SDT) weighing 200–250 g were obtained from the Süddeutsches Tierzuchtinstitut, Tuttingen, Federal Republic of Germany, and fed ad libitum until used. Male X. laevis females were purchased from H. Kahl, Hamburg, FRG and kept under standard conditions as described (16).

Isolation of Rat Liver mRNA—Total RNA from 12 g of rat liver was prepared by a single-step guanidinium thiocyanate/phenol/chloroform extraction procedure (17). mRNA was purified by oligo(dT)-cellulose chromatography (18). For size-fractionation, mRNA samples (150 μg) were heated to 65 °C for 5 min and then loaded on to a linear 6–20% (w/w) sucrose gradient containing 1 mM PIPEs-NaOH, pH 6.4, 5 mM Na₂EDTA, and 0.1% (w/w) Sarkosyl. The gradient was centrifuged for 19 h at 4 °C at 24,000 rpm (80,000 × gₘ) in a Kontron™ TST 28.17 rotor. Thereafter, 17 × 1-ml fractions were collected from the bottom of the tubes. Total and size-fractionated mRNA were precipitated with sodium acetate and ethanol (18), resuspended in water and stored at −80 °C. mRNA concentrations were estimated by measuring the absorption at 260 nm (18).

Expression of mRNA in Xenopus Oocytes—Frogs were anaesthetized by immersion for 15 min in a 0.17% solution of ethyl m-aminobenzoate (MS-222). Oocytes were removed (16) and incubated at room temperature for 3 h in Ca²⁺-free OR-2 solution (19) supplemented with 2 mg/ml collagenase (Sigma type I). They were then washed in modified Barth’s solution consisting of (mM): 88 NaCl, 1 KC1, 24 NaHCO₃, 15 HEPES-NaOH, pH 7.6, 0.3 Ca(NO₃)₂, (H₂O), 0.41 CuCl₂, (H₂O), 0.82 MgSO₄, (H₂O) and containing 10 units/ml penicillin and 10 μg/ml streptomycin, and stage V and VI oocytes were selected. After an overnight incubation at 18 °C in modified Barth’s solution, healthy oocytes were injected with 60 ng of rat liver mRNA (1 mg/ml) or a corresponding volume of H₂O. Subsequently, the expression of the basolateral Na+/bile acid cotransporter was studied in Xenopus laevis oocytes. Injection of rat liver poly(A)+ RNA into X. laevis oocytes resulted in the functional expression of the hepatocellular Na+/bile acid cotransporter within 3–5 days. Furthermore, a 1.5–3.0-kilobase-size-class of mRNA was sufficient to generate the Na+-dependent bile acid (taurocholate) uptake signal. Hence, expression cloning represents a promising approach to ultimately clone the gene of the basolateral Na+/bile acid cotransport system of rat liver.

By guest on October 20, 2017 http://www.jbc.org/ Downloaded from
Expression of Hepatocyte Na⁺/Bile Acid Cotransport

Oocytes were cultured for 3-5 days at 18 °C with a daily change of modified Barth's solution.

**Taurocholate Uptake into Oocytes**—Cultured oocytes were washed once in a Na⁺-free buffer (25 °C) consisting of (in mM): 100 choline chloride, 2 KCl, 1 CaCl₂, 1 MgCl₂, and 10 HEPES-Tris, adjusted to pH 7.5. Between 5 and 10 oocytes were then incubated at 25 °C in 100 μl of uptake medium containing (in mM): 100 NaCl or 100 choline chloride, 2 KCl, 1 CaCl₂, 1 MgCl₂, 10 HEPES-Tris, pH 7.6, and 3-10 μCi of [³H]taurocholate. With the exception of the kinetic uptake studies (i.e. Fig. 2) the concentration of taurocholate in the uptake medium was routinely adjusted to 17 μM. After the indicated time interval, the uptake was terminated by the addition of 1 ml of ice-cold stop solution, which was of similar composition as the Na⁺-free incubation buffer except that 1 mM unlabeled taurocholate was included in order to reduce unspecified binding of tracer taurocholate. Furthermore, oocytes were additionally washed 3 times in 10 ml of cold stop solution. Single oocytes were then dissolved in 1 ml of 10% (w/w) sodium dodecyl sulfate. After addition of 5 ml of scintillation fluid (Opti-Fluor™, Packard Instrument International S.A., Zurich, Switzerland) the oocyte-associated radioactivity was counted in a Packard Tri-Carb™ 2200 CA liquid scintillation analyzer.

**RESULTS AND DISCUSSION**

For adequate interpretation of results obtained with mRNA-injected oocytes, it was first important to exclude the presence of endogenous Na⁺-dependent taurocholate uptake in untreated oocytes. In fact, initial experiments established that cultured control oocytes (injected with 60 ng of H₂O or not injected at all) exhibited very low and similar taurocholate uptake rates in the presence (0.027 ± 0.015 pmol of taurocholate/oocyte/h; mean ± S.D. of a total of 25-50 determinations derived from five different oocyte preparations) and absence (0.022 ± 0.013 pmol of taurocholate/oocyte/h) of an inwardly directed NaCl gradient. These results also demonstrate very low unspecific binding of [³H]taurocholate to the oocyte membrane. In contrast, after 5 days in culture, oocytes injected with 60 ng of rat liver mRNA expressed significant Na⁺ gradient-stimulated taurocholate uptake. As demonstrated in Fig. 1, this Na⁺ gradient-dependent taurocholate uptake increased linearly over at least 4 h and was stimulated 3-7-fold as compared to an inwardly directed choline gradient. Furthermore, inclusion of 1 mM DIDS into the uptake medium completely inhibited the Na⁺ gradient-dependent portion of taurocholate uptake at all time points measured (Fig. 1). These results are similar to the characteristics of taurocholate uptake in the intact liver (19), in isolated hepatocytes (2, 5), and in isolated basolateral rat liver plasma membrane vesicles (4, 20), thus suggesting functional expression of the basolateral Na⁺/taurocholate cotransport system by *X. laevis* mRNA. This assumption was further verified by determination of the kinetics and substrate specificity of the expressed Na⁺ gradient-stimulated taurocholate uptake activity.

As illustrated in Fig. 2, the Na⁺-dependent taurocholate uptake portion was a saturable function of the substrate concentration. If uptake values in the presence of choline were subtracted from values in the presence of Na⁺, the Na⁺-gradient-specific uptake portion demonstrated an apparent *Kₘ* of 91 ± 15 μM and a *Vₘₐₓ* of 6.0 ± 0.4 pmol of taurocholate/oocyte/h (mean ± S.D. of 20 uptake measurements in two separate oocyte preparations). This apparent *Kₘ*, is approximately 2-fold higher than the corresponding values previously found in isolated basolateral rat liver plasma membrane vesicles and in intact hepatocytes (1-4). However, *Kₘ*, values up to 250 μM have also been reported (21, 22). Thus, the kinetic experiments are compatible with a preferential, if not exclu-

**Fig. 2.** Kinetics of Na⁺-dependent taurocholate uptake in mRNA-injected oocytes. Oocytes were treated as described in the legend to Fig. 1. One-h taurocholate uptake measurements (25 °C) were performed in the presence of increasing substrate concentrations with (■) and without (Δ) addition of 100 mM NaCl to the incubation medium. The uptake values represent the mean ± S.D. of 10-20 determinations in two separate oocyte preparations. The curves were fitted by a computer-based nonlinear regression analysis.

**Fig. 3.** Effects of various organic anions and of bovine serum albumin (BSA) on Na⁺-dependent taurocholate uptake in mRNA-injected oocytes. Oocytes were treated as described in the legend to Fig. 1. Na⁺-gradient-stimulated taurocholate uptakes were determined at 25 °C for 1 h in the presence and in the absence (control = 100%) of the indicated concentrations of the various compounds. The given uptake values represent the mean ± S.D. of 10-20 determinations in two separate oocyte preparations. PAH, p-aminohippurate; BSP, bromosulfophthalein.
taurocholate uptake activity. As demonstrated in Fig. 3, the anion transport inhibitors bromosulfophthalein inhibited the Na+-dependent uptake of rochenodeoxycholate, the synthetic keto-bile acid taurodehydrocholate as well as p-aminobenzoic acid. Furthermore, as previously shown in intact liver (23) and in isolated basolateral rat liver plasma membrane vesicles (4, 10), the expressed Na+-dependent taurocholate uptake system indeed corresponds to the basolateral Na'/bile acid cotransport system in intact hepatocytes and in isolated rat liver plasma membrane vesicles. Thus, the identified Na' gradient. The mRNA from each fraction was precipitated, washed, and resuspended in H2O. Fifty-nl samples were injected into oocytes. After three days in culture the oocytes were used for taurocholate uptake measurements in the presence (closed bars) and in the absence (hatched bars) of 100 mM NaCl in the outside medium. The uptake values represent the mean ± S.D. of 5–10 determinations of one out of two oocyte preparations.

The present study demonstrates that injection of total rat liver mRNA into X. laevis oocytes results in functional expression of Na+-dependent taurocholate uptake activity with characteristic properties of the basolateral Na+/ bile acid cotransport system in intact hepatocytes and in isolated rat liver plasma membrane vesicles. Thus, the identified Na' gradient. The mRNA from each fraction was precipitated, washed, and resuspended in H2O. Fifty-nl samples were injected into oocytes. After three days in culture the oocytes were used for taurocholate uptake measurements in the presence (closed bars) and in the absence (hatched bars) of 100 mM NaCl in the outside medium. The uptake values represent the mean ± S.D. of 5–10 determinations of one out of two oocyte preparations.

Fig. 4. Fractionation of the mRNA on a linear sucrose density gradient. 150 μg of total rat liver mRNA were size-fractionated on a linear 6–20% (w/w) sucrose gradient. After centrifugation 17 × 1-ml fractions were collected and the mRNA from each fraction precipitated, washed, and resuspended in H2O. Fifty-nl samples were injected into oocytes. After three days in culture the oocytes were used for taurocholate uptake measurements (1-h values) in the presence (closed bars) and in the absence (hatched bars) of 100 mM NaCl in the outside medium. The uptake values represent the mean ± S.D. of 5–10 determinations of one out of two oocyte preparations.

Finally, total rat liver mRNA was size-fractionated on linear sucrose gradient in order to enrich the mRNA species encoding the Na+-dependent taurocholate uptake system. As shown in Fig. 4, the most active mRNA was found within an 18 S subfraction, which, based on additional agarose gel electrophoresis (data not shown), corresponded to a mRNA size-class between 1.5 and 3.0 kilobases. Hence, assuming an average molecular weight of 120 for amino acids, the 18 S subfraction of the mRNA could well account for expression of the 49-kDa polypeptide suggested to be directly involved in Na+-dependent uptake of bile acids into rat hepatocytes (5).

In conclusion, the present study demonstrates that injection of total rat liver mRNA into X. laevis oocytes results in functional expression of Na+-dependent taurocholate uptake activity with characteristic properties of the basolateral Na+/ bile acid cotransport system in intact hepatocytes and in isolated rat liver plasma membrane vesicles. Thus, the identified Na' gradient. The mRNA from each fraction was precipitated, washed, and resuspended in H2O. Fifty-nl samples were injected into oocytes. After three days in culture the oocytes were used for taurocholate uptake measurements (1-h values) in the presence (closed bars) and in the absence (hatched bars) of 100 mM NaCl in the outside medium. The uptake values represent the mean ± S.D. of 5–10 determinations of one out of two oocyte preparations.

REFERENCES
Expression of Hepatocyte Na⁺/Bile Acid Cotransport

Expression of the hepatocyte Na+/bile acid cotransporter in Xenopus laevis oocytes.

B Hagenbuch, H Lübbert, B Stieger and P J Meier


Access the most updated version of this article at http://www.jbc.org/content/265/10/5357

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/10/5357.full.html#ref-list-1