

Expression of Rat Liver Cell Membrane Transporters for Thyroid Hormone in *Xenopus laevis* Oocytes*

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ABSTRACT

The present study was conducted to explore the possible use of *Xenopus laevis* oocytes for the expression cloning of cell membrane transporters for iodothyronines. Injection of stage V–VI *X. laevis* oocytes with 23 ng Wistar rat liver polyadenylated RNA (mRNA) resulted after 3–4 days in a highly significant increase in [¹²⁵I]T₃ (5 nM) uptake from 6.4 ± 0.8 fmol/oocyte·h in water-injected oocytes to 9.2 ± 0.65 fmol/oocyte·h (mean ± SEM; n = 19). In contrast, [¹²⁵I]T₄ (4 nM) uptake was not significantly stimulated by injection of total liver mRNA. T₃ uptake induced by liver mRNA was significantly inhibited by replacement of Na⁺ in the incubation medium by choline⁺ or by simultaneous incubation with 1 μM unlabeled T₃. In contrast, T₃ uptake by water-injected oocytes was not Na⁺ dependent. Fractionation of liver mRNA on a 6–20% sucrose gradient showed that maximal stimulation of T₃ uptake was obtained with mRNA of 0.8–2.1 kilobases (kb). In contrast to unfractionated mRNA, the 0.7- to 2.1-kb fraction also significantly stimulated transport of T₄, and it

was found to induce uptake of T₃ sulfate (T₃S). Because T₃S is a good substrate for type I deiodinase (D1), 2.3 ng rat D1 complementary RNA (cRNA) were injected either alone or together with 23 ng of the 0.8- to 2.1-kb fraction of rat liver mRNA. Compared with water-injected oocytes, injection of D1 cRNA alone did not stimulate uptake of [¹²⁵I]T₃S (1.25 nM). T₃S uptake in liver mRNA and D1 cRNA-injected oocytes was similar to that in oocytes injected with mRNA alone, showing that transport of T₃S is independent of the metabolic capacity of the oocyte. Furthermore, coinjection of liver mRNA and D1 cRNA strongly increased the production of ¹²⁵I⁻, showing that the T₃S taken up by the oocyte is indeed transported to the cell interior.

In conclusion, injection of rat liver mRNA into *X. laevis* oocytes resulted in a stimulation of saturable, Na⁺-dependent T₄, T₃ and T₃S transport, indicating that rat liver contains mRNA(s) coding for plasma membrane transporters for these iodothyronine derivatives. (*Endocrinology* 138: 1841–1846, 1997)

T₄ IS THE main secretory product of the thyroid gland, which is enzymatically converted in peripheral tissues to the biologically active hormone T₃. About 80% of the plasma T₃ production in man results from this extrathyroidal pathway, in which the liver plays a dominant role (1). The conversion of T₄ to T₃ as well as the further deiodination of iodothyronines is effected by different types of deiodinases in tissues (2). Transport across the plasma membrane is required for intracellular deiodination. During the last 15 yr evidence has accumulated that the plasma membranes of different tissues contain one or more specific transport proteins for T₃ and T₄. The transport process appears to be temperature and energy dependent, and is inhibited by hormone analogs and compounds that disturb the Na⁺ gradient across the plasma membrane (see Refs. 3 and 4 for comprehensive reviews). Although much is known about the physiology of transmembrane T₄ and T₃ transport, little is known about the molecular mechanisms of these processes. Studies with a monoclonal antibody that inhibits T₄ and T₃ transport into rat hepatocytes showed immunoprecipitation of a plasma membrane protein from rat liver with a M_r of about

55 kDa (5). In another study, photoaffinity labeling of rat erythrocyte membranes with [¹²⁵I]T₃ resulted in the identification of a 45-kDa protein (6). Additional studies using the above monoclonal antibody and other inhibitors of plasma membrane transport also indicated that this transport process is rate limiting for subsequent metabolism of thyroid hormone (7). Other workers have shown that this transport process is a determinant for the nuclear occupancy of thyroid hormone (8, 9). Thus, plasma membrane transport may play an important role in the overall regulation of thyroid hormone bioactivity.

No information is as yet available about the structure of this transport protein(s). During the last years a large number of plasma membrane transporters (for instance, for amino acids, organic anions, bile acid, sulfate, and water) have been cloned following their expression in *Xenopus laevis* oocytes after microinjection of messenger RNA (mRNA) coding for these proteins (10–16). Therefore, we have adopted this technique to express the thyroid hormone transport protein(s) using total rat liver polyadenylated [poly(A)⁺] RNA (mRNA) and fractions thereof as a first step in the cloning process.

Materials and Methods

Materials

T₃ was purchased from Sigma Chemical Co. (St. Louis, MO), [3',5'-¹²⁵I]T₄ (>19 MBq/nmol), [3'-¹²⁵I]T₃ (>29 MBq/nmol), and L-[³H]arginine (>2.22 MBq/nmol) were obtained from RCC Amersham (Aylesbury, UK). [3'-¹²⁵I]T₃ sulfate ([¹²⁵I]T₃S) was prepared by reaction of [¹²⁵I]T₃ with chlorosulfonic acid in dimethylformamide (both from

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Merck, Darmstadt, Germany) and purified by Sephadex LH-20 (Pharmacia Fine Chemicals, Uppsala, Sweden) chromatography as previously described (17). [¹²⁵I]T₄ was purified before use with the same method. All other chemicals were of reagent grade.

Animals

Two- to 3-yr-old adult *Xenopus laevis* females were obtained from the Hubrecht Laboratory (Utrecht, The Netherlands). They were maintained in a water-filled tank with three dark sides at a temperature of 18–22 C. A 12-h light, 12-h dark cycle was maintained to reduce seasonal variations in oocyte quality. Frogs were fed twice a week. The water was changed about 2 h after feeding.

Livers of male Wistar rats, female Sprague-Dawley rats, and female Fisher rats were used to prepare mRNA. Animals had free access to food and water and were kept in a controlled environment (21 C) with constant day length (12 h).

Oocyte isolation and RNA injections

Oocytes were prepared as described previously (18), with some modifications. Ovarian fragments were removed from *X. laevis* females under MS-222 anesthesia (Sigma; 1 g/liter 3-aminobenzoic acid ethyl ester, in tap water) and hypothermia. Small lumps containing 20–50 oocytes were washed in calcium-free ORII (82 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 10 mM HEPES, and 10 mM Tris, pH 7.5). To remove follicular layers, the lumps were incubated twice for 90 min each time at room temperature in ORII with 2 mg/ml collagenase A (Boehringer Mannheim, Mannheim, Germany) on a rotator. The oocytes were washed thoroughly five times with ORII and subsequently five times with MBS [modified Barth's solution, 88 mM NaCl, 1 mM KCl, 0.82 mM MgSO₄, 0.4 mM CaCl₂, 0.33 mM Ca(NO₃)₂, 2.4 mM NaHCO₃, 10 mM HEPES (pH 7.4), containing 10 IU/ml penicillin, and 10 μg/ml streptomycin]. The oocytes were sorted manually on morphological criteria, such as size, polarization, pigmentation, and absence of follicular layer debris. Healthy-looking stage V–VI oocytes (19) were transferred to six-well tissue culture plates and incubated in MBS at 18 C in the dark.

The next day, oocytes were injected with 0.23–23 ng RNA in 23 nl water or with water alone (control) using a Nanoject system (Drummond Scientific Co., Broomall, PA). Injected oocytes were maintained in MBS at 18 C for 3–4 days, with a daily change of medium.

mRNA isolation

A commercial kit (Stratagene, La Jolla, CA) was used for the isolation of mRNA from rat liver tissue according to the manufacturer's protocol. Tissue was homogenized in guanidinium isothiocyanate buffer with β-mercaptoethanol. After dilution, precipitated proteins were removed by centrifugation, and the mRNA was bound to oligo(deoxythymidine)cellulose. After several wash steps, mRNA was eluted with elution buffer at 65 C. For size-fractionation, rat liver mRNA (150 μg) in water was heated to 65 C for 5 min and then loaded on a linear 6–20% (wt/vol) sucrose gradient containing 15 mM piperazine-*N,N'*-[2-ethanesulfonic acid] (PIPES) (pH 6.4), 5 mM Na₂-EDTA, and 0.25% (wt/vol) Sarkosyl. The gradient was centrifuged for 19 h at 4 C at 25,000 rpm (80,000 × g_{av}) in a Beckman SW 41 rotor (Beckman, Palo Alto, CA). Subsequently, 0.7-ml fractions were collected from the bottom of the tubes. Total and size-fractionated mRNA were precipitated with 0.3 M sodium acetate in ethanol (20), resuspended in water at a concentration of 1 μg/μl, and stored at –80 C. mRNA concentrations were estimated by measuring the absorption at 260 nm (20). The size ranges of mRNAs in each fraction were estimated by electrophoresis of the fractions on 1% agarose gel and staining with ethidium bromide. Each fraction contains a maximum concentration of one size of mRNAs (mRNA_{max}) with gradually lower concentrations of smaller and larger species of mRNA, extending about 0.6 kilobase (kb) on each side of the mRNA_{max}. To combine data from different gradient experiments, results were grouped according to the mRNA_{max} in each fraction, *i.e.* less than 0.5, 0.5–1.5, 1.5–2.5, and more than 2.5 kb, respectively.

In vitro transcription

Complementary RNA (cRNA) coding for the arginine (Arg) transporter rBAT (16) or for rat type I deiodinase (D1) (21) was prepared by

in vitro transcription using the AmpliScribe T3 transcription kit (Epicentre Technologies, Madison, WI) according to the protocol for synthesis of capped cRNA. For capping, the m7G[5']ppp[5']G cap analog was used (Epicentre Technologies). pBluescript DNAs containing the respective complementary DNAs (cDNAs) as insert were used as template after linearization with *Xho*I (Boehringer Mannheim). After transcription, the DNA template was digested using ribonuclease-free deoxyribonuclease I, and the incubation mixture was extracted twice with an equal volume of phenol-chloroform-isoamyl alcohol (Life Technologies, Breda, The Netherlands) and once with chloroform. The cRNA in the final water phase was precipitated with an equal volume of 5 M ammonium acetate, incubated on ice for 30 min, and centrifuged for 10 min at 4 C. cRNA pellets were dissolved in water and stored at –80 C.

Uptake assays

Groups of 10 oocytes were washed for 1 min at 18 C in choline⁺-containing incubation buffer (100 mM choline chloride, 2 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM HEPES, and 10 mM Tris, pH 7.5). Subsequently, the oocytes were incubated for 1 h at 25 C in 0.1 ml of the same buffer containing 50 μM Arg and 370 kBq/ml [³H]Arg or with 4 nM [¹²⁵I]T₄ (60 kBq/ml), 5 nM [¹²⁵I]T₃ (60 kBq/ml), or 1.25 nM [¹²⁵I]T₃ sulfate ([¹²⁵I]T₃S; 90 kBq/ml). Uptake of these labeled compounds was also tested in Na⁺ buffer (same buffer with 100 mM NaCl instead of choline chloride) to assess Na⁺-dependent uptake. After 1 h, incubation buffer was removed, and the oocytes were washed four times with 2.5 ml ice-cold Na⁺ buffer containing 0.1% BSA. Oocytes were transferred to new tubes or scintillation vials and counted individually.

Metabolism assays

Groups of 10 oocytes were transferred to a 96-well tissue culture plate. Subsequently, the oocytes were incubated at 18 C in the dark in 0.1 ml MBS containing 1.25 nM [¹²⁵I]T₃S (90 kBq/ml). After 18 h, the incubation medium was removed, and the oocytes were transferred to tubes and washed 4 times with 2.5 ml ice-cold Na⁺ buffer containing 0.1% BSA. Each group of 10 oocytes was divided into 2 groups of 5 oocytes, transferred to new tubes, counted, homogenized in 0.1 ml 0.1 M NaOH, and centrifuged. The supernatants were analyzed for ¹²⁵I[–], [¹²⁵I]T₃S, and [¹²⁵I]T₃ by Sephadex LH-20 chromatography as previously described (22).

Statistics

Data are presented as the mean ± SEM. Statistical significance was evaluated by Student's *t* test for unpaired observations. Data from the gradient experiments were analyzed by ANOVA, using the Studentized range for comparison of group means.

Results

Injection of 0.23 ng rBAT cRNA, which expresses Na⁺-independent transport of L-arginine, L-cysteine, and L-leucine (16), resulted in a rise in [³H]Arg uptake from 10 (water-injected) to 205 pmol/oocyte·h in choline⁺-containing medium (Table 1) without a change in T₃ uptake in both the presence and absence of Na⁺. Injection of 23 ng total liver mRNA resulted in a moderate, but significant (*P* < 0.001), increase in T₃ uptake from 6.4 ± 0.8 (water-injected) to 9.2 ± 0.65 fmol/oocyte·h (Table 1). This increase was completely blocked by replacing Na⁺ in the uptake medium by choline⁺, indicating that the increase in T₃ uptake is Na⁺ dependent. From the data in Table 1, it is also clear that water-injected oocytes exhibited an endogenous T₃ uptake that was not significantly inhibited by replacement of Na⁺ by choline⁺. Furthermore, it appeared that this endogenous uptake was highly dependent on the batch of oocytes used (range, 1.7–13.9 fmol T₃/oocyte·h). To eliminate this variable endogenous uptake, we have taken the difference in uptake between

TABLE 1. Uptake of T₃ and arginine by *X. laevis* oocytes

Injected material	Uptake ^a		
	T ₃ (Na ⁺) (fmol/oocyte)	T ₃ (choline ⁺) (fmol/oocyte)	Arg (choline ⁺) (pmol/oocyte)
Water	6.4 ± 0.8	5.0 ± 0.5	10 ± 1.8
0.23 ng rBAT cRNA	6.5 ± 0.7	5.1 ± 0.4	205 ± 18.7 ^b
23 ng liver mRNA	9.2 ± 0.65 ^c	5.5 ± 0.5	

Results represent the mean ± SEM of 19 (T₃) or 14 (Arg) experiments.

^a Ten oocytes injected 4 days previously with water, rBAT cRNA, or Wistar rat liver mRNA were incubated for 1 h with 5 nM [¹²⁵I]T₃ or 50 μM [³H]Arg in 0.1 ml medium with Na⁺ or choline⁺.

^b *P* < 0.001 vs. water-injected oocytes.

^c *P* < 0.01 vs. water-injected oocytes.

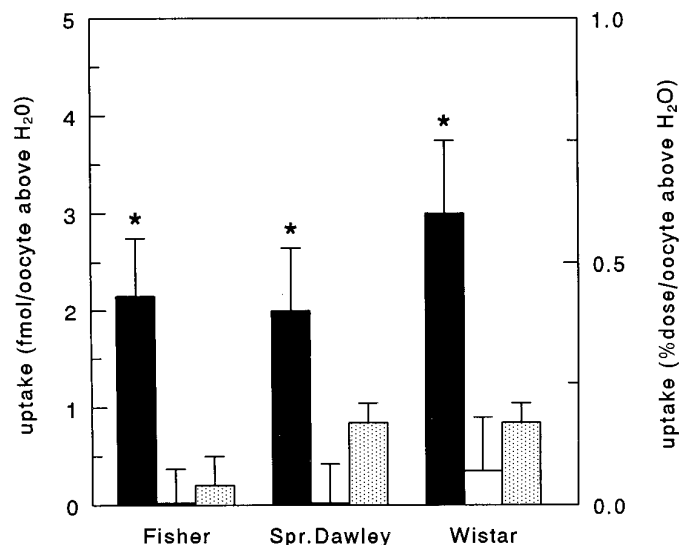


FIG. 1. Induction of T₃ uptake by oocytes after injection of total liver mRNA from different rat strains. Groups of 10 oocytes were injected with 23 ng/oocyte liver mRNA or with water, and after 4 days they were incubated for 1 h at 25 C with 5 nM [¹²⁵I]T₃ in 0.1 ml medium containing Na⁺ (■), choline⁺ (□), or Na⁺ and 1 μM unlabeled T₃ (▨). The results show the difference between T₃ uptake by mRNA-injected oocytes and that by water-injected oocytes, and are presented as the mean ± SEM of 10 oocytes. *Left y-axis*, Uptake of [¹²⁵I]T₃ in femtomoles per oocyte/h; *right y-axis*, uptake as a percentage of the added [¹²⁵I]T₃. *, *P* < 0.001 vs. zero.

mRNA-injected and water-injected oocytes in each further experiment as a measure of mRNA-induced T₃ uptake.

Figure 1 shows the induction of T₃ uptake by oocytes injected with 23 ng mRNA prepared from livers of three different rat strains and sexes. No significant difference was found among the preparations. mRNA-induced T₃ uptake in choline⁺-containing medium was not significantly different from zero, indicating that the expressed transport system is completely Na⁺ dependent. Furthermore, addition of 1 μM unlabeled T₃ inhibited the uptake of [¹²⁵I]T₃ by more than 50%.

Total Sprague-Dawley rat liver mRNA was size-fractionated on a 6–20% sucrose gradient, and the mRNA size ranges were determined by agarose gel electrophoresis. Of each fraction, 23 ng mRNA were injected into oocytes. The fractions containing mRNA of 0.8–2.1 kb showed the largest stimulation of Na⁺-dependent T₃ uptake. A typical experiment is depicted in Fig. 2.

In 9 fractionation experiments, water-injected oocytes

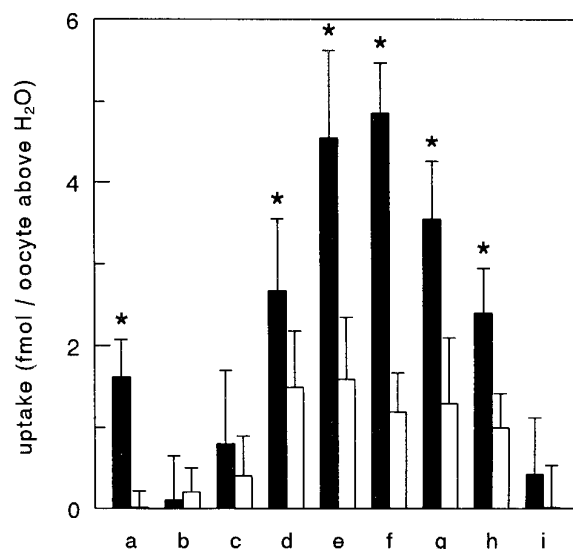


FIG. 2. Induction of T₃ uptake by oocytes after injection of various size fractions of rat liver mRNA. Groups of 10 oocytes were injected with 23 ng/oocyte liver mRNA or with water, and after 4 days they were incubated for 1 h at 25 C with 5 nM [¹²⁵I]T₃ in 0.1 ml medium containing Na⁺ (■) or choline⁺ (□). The results show the difference between T₃ uptake by mRNA-injected oocytes and that by water-injected oocytes, and are presented as the mean ± SEM of 10 oocytes. Size ranges of RNA fractions: a, unfractionated mRNA; b, 3.0–4.5; c, 2.2–3.5; d, 1.5–3 kb; e, 1.25–2.5 kb; f, 0.8–2.1 kb; g, 0.5–1.4 kb; h, 0.3–1.25 kb; and i, 0.2–0.9 kb. *, *P* < 0.001 vs. zero.

showed a T₄ uptake of 3.7 ± 0.7 fmol/oocyte·h and a T₃ uptake of 7.0 ± 1.3 fmol/oocyte·h. Injection of 23 ng total liver mRNA did not stimulate the uptake of T₄ significantly. mRNA-induced uptake of T₄ amounted to only 0.12 ± 0.08 fmol/oocyte·h, whereas mRNA-induced T₃ uptake in the same experiments was 0.95 ± 0.16 fmol/oocyte·h (*P* < 0.001; Fig. 3a). However, after fractionation of rat liver mRNA on a 6–20% sucrose gradient, uptake of T₄ was also significantly induced, in particular with mRNA of 0.5–2.5 kb, similar to T₃ uptake (Fig. 3, c and d).

Figure 4 shows that uptake of T₃S during 1 h was stimulated by injection of oocytes with a 0.8- to 2.1-kb fraction of rat liver mRNA, and that this uptake was Na⁺ dependent. mRNA-induced T₃S uptake was inhibited by 50% in the presence of 1 μM unlabeled T₃S (data not shown). As basal uptake of T₃S by water-injected oocytes was low, injection of rat liver mRNA produced a relatively much larger signal compared with the effect on T₃ uptake. Injection of 2.3 ng D1 cRNA did not stimulate T₃S uptake, whereas injection of a

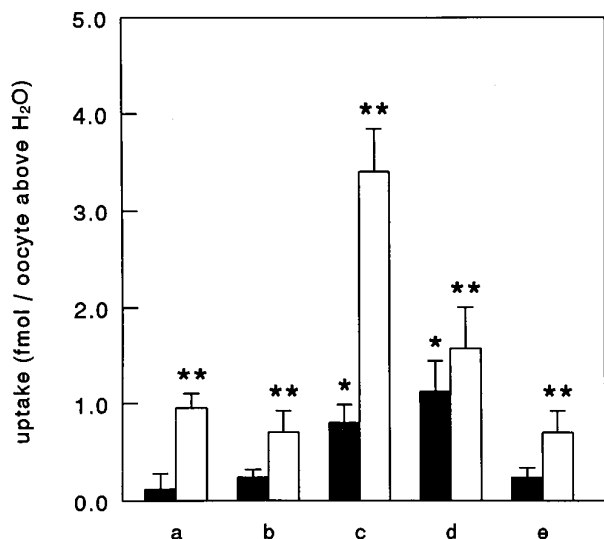


FIG. 3. Induction of T₄ (■) and T₃ (□) uptake by oocytes after injection of various size fractions of rat liver mRNA. Groups of 10 oocytes were injected with 23 ng/oocyte liver mRNA or with water, and after 4 days they were incubated for 1 h at 25 C with 5 nM [¹²⁵I]T₃ or 4 nM [¹²⁵I]T₄ in 0.1 ml Na⁺ medium. The results show the difference between T₃ uptake by mRNA-injected oocytes and that by water-injected oocytes and are presented as the mean ± SEM of nine experiments. Size ranges of RNA fractions: a, unfractionated mRNA; b, mRNA_{max} less than 0.5 kb; c, mRNA_{max} 0.5–1.5 kb; d, mRNA_{max} 1.5–2.5 kb; e, mRNA_{max} more than 2.5 kb. c vs. b: T₄, *P* < 0.05; T₃, *P* < 0.001; c vs. d: T₄, *P* = NS; T₃, *P* < 0.01; d vs. e: T₄, *P* < 0.01; T₃, *P* = NS. **, *P* < 0.001; *, *P* < 0.01 (vs. zero).

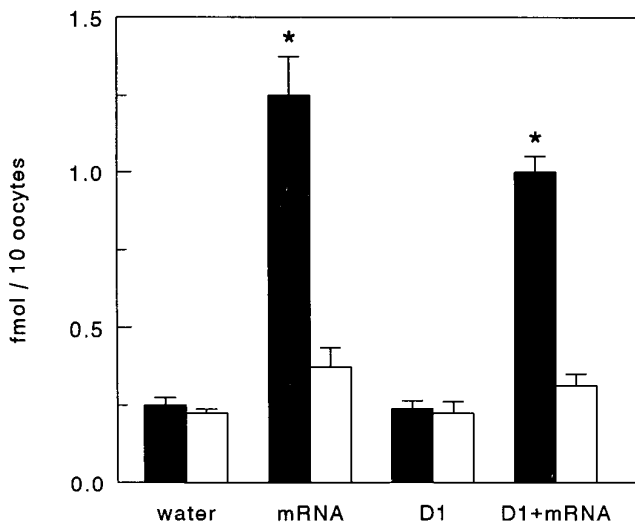


FIG. 4. T₃S uptake by oocytes injected with water, 0.8–2.1 kb rat liver mRNA, D1 cRNA, or the combination of liver mRNA and D1 cRNA. Groups of 10 oocytes were injected with 23 ng/oocyte mRNA, 2.3 ng/oocyte D1 cRNA, or both, and after 4 days they were incubated for 1 h at 25 C with 1.25 nM [¹²⁵I]T₃S in 0.1 ml medium containing Na⁺ (■) or choline⁺ (□). Data are presented as the mean ± SEM of 10 oocytes. *, *P* < 0.001 vs. water-injected oocytes.

mixture of 0.8–2.1 kb liver mRNA and D1 cRNA stimulated T₃S uptake to a similar extent as liver mRNA alone. Figure 5 shows the results of prolonged incubations (18 h) of similarly injected oocytes with [¹²⁵I]T₃S. Total T₃S uptake after 18 h was obviously higher than with 1-h incubations (Fig. 4).

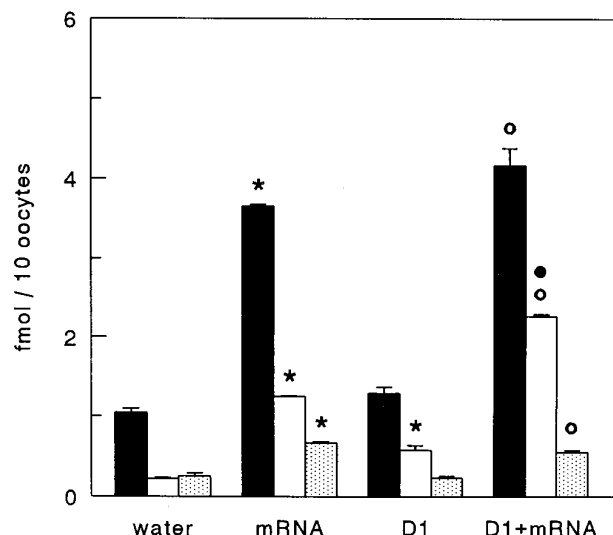


FIG. 5. Metabolism of T₃S in oocytes injected with water, 0.8–2.1 kb rat liver mRNA, D1 cRNA, or the combination of liver mRNA and D1 cRNA. Groups of 10 oocytes were injected with water, 23 ng/oocyte mRNA, 2.3 ng/oocyte D1 cRNA, or both, and after 4 days they were incubated for 18 h at 18 C with 1.25 nM [¹²⁵I]T₃S in 0.1 ml Na⁺ medium. Results show the total amount of radioactivity (■), I⁻ (□), and T₃ (▨) in the oocytes at the end of the incubation and are presented as the mean ± SEM of four pools of five oocytes. *, *P* < 0.001 vs. water-injected oocytes; o, *P* < 0.001 vs. D1 cRNA- or water-injected oocytes; ●, *P* < 0.001 vs. liver mRNA-injected oocytes.

Total T₃S uptake was low in water- or D1 cRNA-injected oocytes, but was strongly stimulated in oocytes injected with 0.8–2.1 kb liver mRNA. Total T₃S uptake by oocytes injected with liver mRNA together with D1 cRNA was similar to uptake of oocytes injected with rat liver mRNA alone. In water-injected oocytes, deiodination of T₃S and deconjugation of T₃S to T₃ were very low. Deconjugation was similarly low in D1 cRNA-injected oocytes, but deiodination of T₃S was stimulated by expression of D1 (*P* < 0.01). In oocytes injected with liver mRNA, more T₃S was hydrolyzed than in water- or D1 cRNA-injected oocytes (*P* < 0.001), and T₃S was also deiodinated to some extent (*P* < 0.001). Finally, injection of oocytes with the combination of liver mRNA and D1 cRNA led to a much larger production of iodide from T₃S than injection of either RNA alone (*P* < 0.001).

Discussion

Our present findings that Na⁺-dependent uptake of T₃, T₄, and T₃S is induced in *X. laevis* oocytes by the injection of rat liver mRNA are in agreement with our previous suggestion that transport of iodothyronines into rat hepatocytes proceeds via a plasma membrane transporter, as it is a saturable process dependent on temperature, the intracellular ATP concentration, and the Na⁺ gradient over the cell membrane (3, 23). Because the oocytes exhibit an endogenous uptake system for T₃ which is independent of the Na⁺ gradient, we have defined mRNA-induced T₃ uptake as the difference between T₃ uptake by mRNA-injected oocytes and that by water-injected oocytes in the same medium. Our finding that mRNA-induced T₃ uptake is completely abolished by replacement of Na⁺ in the uptake medium by choline⁺ strongly suggests that this uptake is tightly coupled to the

Na⁺ gradient. This may also explain the ATP dependence of uptake of T₄ and T₃ by hepatocytes, because ATP is necessary to maintain the Na⁺ gradient over the plasma membrane (24, 25). Livers from different rat strains contain similar amounts of mRNA coding for the T₃ transporter, showing the same Na⁺ dependence and similar saturability with unlabeled T₃.

Uptake of T₄, T₃, and T₃S by oocytes is induced by the injection of certain mRNA species, but not by others. Thus, T₃ uptake by oocytes injected with rBAT cRNA is not different from that by water-injected oocytes. Similarly, injection of D1 cRNA does not induce T₃S uptake. Furthermore, not all rat liver mRNA fractions tested stimulate T₃ uptake; mRNAs of 0.2–0.9 and 2.2–4.5 kb have no effect, but mRNA of 0.8–2.1 kb induces a 2.5-fold greater stimulation of T₃ uptake than crude rat liver mRNA. Taken together, our results suggest that the size of the mRNA coding for the T₃ transporter is 1.5 ± 0.5 kb, a size large enough to code for a protein of 45–55 kDa, previously estimated by immunoprecipitation and photoaffinity labeling of putative cellular T₃ transport proteins (5, 6).

Induction of T₄ uptake in oocytes is undetectable after the injection of total rat liver mRNA. However, when liver mRNA is fractionated on a 6–20% sucrose gradient, a highly significant stimulation is found in fractions with mRNA_{max} ranging between 0.5–2.5 kb. This stimulation is significantly higher than that by fractions with mRNA_{max} smaller than 0.5 kb or larger than 2.5 kb. Although the stimulation of T₄ uptake seems somewhat larger in fractions in which mRNA_{max} ranges between 1.5–2.5 kb, there is no significant difference from that in the fractions in the 0.5–1.5 kb size range. The largest stimulation of T₃ uptake was found in fractions containing mRNA_{max} of 0.5–1.5, significantly higher than the smaller and larger mRNA size fractions. Therefore, stimulation of T₄ and T₃ uptake seems to peak in different mRNA fractions, suggesting that T₄ and T₃ transporters are translated from different mRNAs. This is in agreement with previous indirect evidence, suggesting different mechanisms for T₃ and T₄ uptake in liver cell membranes. Uptake of T₄ is lower than that of T₃, which is also in accordance with previous findings using rat hepatocytes (26), indicating that the V_{max} for the specific uptake of T₄ was 3.5-fold lower than that for T₃ uptake.

Uptake of T₃ and T₃S by oocytes is linear during the first hour (data not shown), suggesting that the 1-h uptake data represent binding and/or transport at the level of the cell membrane. Although the Na⁺ dependence of the initial T₃ uptake process strongly suggests that this represents transmembrane transport, subsequent metabolism of T₃ would be an unequivocal indication that the hormone indeed enters the oocytes. Unfortunately, T₃ is not metabolized by oocytes. Induction of D1 by injection of its cRNA (21) does not change the situation, because T₃ is a poor substrate for this enzyme. On the other hand, [3'-¹²⁵I]T₃S is rapidly deiodinated by D1, initially in the inner ring and subsequently in the outer ring with liberation of ¹²⁵I⁻ (27). Our results indicate that the relative increase of T₃S uptake by *X. laevis* oocytes after the injection of rat liver mRNA is much greater than that in T₃, although the absolute rate of T₃S uptake is less than that of T₃ even in mRNA-injected oocytes. A similar difference was found between T₃ and T₃S uptake in rat hepatocytes (28). Like

T₃ uptake, liver mRNA-induced T₃S uptake by oocytes is Na⁺ dependent, whereas endogenous T₃S uptake by water-injected oocytes is Na⁺ independent. The finding that deiodination of T₃S is highest in oocytes coinjected with D1 cRNA and rat liver mRNA indicates that T₃S is indeed transported to the cell interior as D1 is an intracellular membrane protein (29). Furthermore, it is clear that uptake of T₃S in mRNA plus D1 cRNA-injected oocytes is similar to that in oocytes injected with mRNA alone. This indicates that plasma membrane transport is independent of the metabolic capacity of the oocyte, underlining the rate-limiting potential of the transport process for entry and subsequent metabolism of thyroid hormone (7).

In conclusion, we present a system for expression cloning of cDNA coding for rat liver T₄, T₃, and T₃S transporter(s) based on the 0.8- to 2.1-kb mRNA fraction. This technique may lead to the molecular characterization of thyroid hormone plasma membrane transport proteins and to a better understanding of the molecular mechanism of translocation of thyroid hormone across the plasma membrane of target cells.

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