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Functional Expression of Mammalian Glucose Transporters in *Xenopus laevis* Oocytes: Evidence for Cell-Dependent Insulin Sensitivity

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We report the functional expression of two different mammalian facilitative glucose transporters in *Xenopus* oocytes. The RNAs encoding the rat brain and liver glucose transporters were transcribed in vitro and microinjected into *Xenopus* oocytes. Microinjected cells showed a marked increase in 2-deoxy-D-glucose uptake as compared with controls injected with water. 2-Deoxy-D-glucose uptake increased during the 5 days after microinjection of the RNAs, and the microinjected RNAs were stable for at least 3 days. The expression of functional glucose transporters was dependent on the amount of RNA injected. The oocyte-expressed transporters could be immunoprecipitated with anti-brain and anti-liver glucose transporter-specific antibodies. Uninjected oocytes expressed an endogenous transporter that appeared to be stereospecific and inhibitable by cytochalasin B. This transporter was kinetically and immunologically distinguishable from both rat brain and liver glucose transporters. The uniqueness of this transporter was confirmed by Northern (RNA) blot analysis. The endogenous oocyte transporter was responsive to insulin and to insulinlike growth factor I. Most interestingly, both the rat brain and liver glucose transporters, which were not insulin sensitive in the tissues from which they were cloned, responded to insulin in the oocyte similarly to the endogenous oocyte transporter. These data suggest that the insulin responsiveness of a given glucose transporter depends on the type of cell in which the protein is expressed. The expression of hexose transporters in the microinjected oocytes may help to identify tissue-specific molecules involved in hormonal alterations in hexose transport activity.

Facilitated glucose uptake in most mammalian cells is mediated by a family of specific integral membrane proteins that catalyze glucose movement down a concentration gradient (45). The cDNAs encoding the glucose transporter expressed in a human hepatoma-derived cell line (HepG2) and rat brain have been isolated and characterized (4, 31). The amino acid sequence of the rat brain glucose transport protein is 98% identical to that of the HepG2 protein and appears to be the same protein as that expressed in human erythrocytes (4, 31). The facilitative glucose carrier of the human erythrocyte is a 55-kilodalton integral membrane glycoprotein that has been well studied. It has been purified and reconstituted in proteoliposomes (24, 45).

The existence of additional facilitative glucose transport proteins has been established with the cloning of cDNAs encoding transporters expressed in liver cells, muscle cells, and adipocytes (15, 21, 26, 41). In addition, RNA blotting analyses indicate a variable degree of expression of transporters in different tissues, with some cells expressing two or more of these proteins (4, 15, 21, 26, 31, 41). The different transporters have homologous amino acid sequences and are predicted to have similar secondary structures (4, 15, 21, 26, 31, 41).

Despite our knowledge of the amino acid sequence of a number of different glucose transport proteins, little is known about the molecular mechanisms involved in the hormonal regulation of facilitated glucose transport. For example, it has been demonstrated that insulin activation of glucose uptake in adipocytes and muscle cells is accompanied by translocation of an intracellular pool of glucose transporters to the cell membrane (5, 10, 40). However, although the cDNA for an insulin-regulatable glucose transporter present in adult skeletal muscle and adipocytes has been cloned (21), we do not know what factors determine the translocation of a specific transporter and how the insulin signal is effected. An approach to this problem requires the development of experimental systems that permit functional expression of chimeric and mutant transporter genes containing sequences derived from different forms of the transporter. As an initial step in that direction, we have expressed the rat brain and liver glucose transporters in *Xenopus* oocytes. The results presented in this communication indicate that both transporters, when expressed in *Xenopus* oocytes, exhibit insulin-stimulated translocation and activation to about the same degree as does the endogenous oocyte transporter.

MATERIALS AND METHODS

Plasmids. The cDNA encoding the rat brain glucose transporter has been described (4). Full-length liver glucose transporter cDNA (41) was kindly provided by B. Thorens and H. Lodish of the Whitehead Institute for Biomedical Research (Cambridge, Mass.).

In vitro transcription. Plasmids containing the appropriate glucose transporter cDNA were linearized with either *Sal*I (brain glucose transporter) or *Mlu*I (liver glucose transporter) according to the recommendations of the supplier. The DNAs were recovered by phenol extraction followed by ethanol precipitation and were used for in vitro transcription and capping, using T7 RNA polymerase (32). Trace amounts of [α-^{32}P]UTP (800 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) were included in the reaction mixture for quantitation of reaction yields. The RNAs were recovered by ethanol precipitation and dissolved in distilled water for injection into oocytes. Typically, 2 to 3 μg of capped RNA was obtained per μg of DNA.

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Preparation of oocytes and RNA injection. Ovaries were removed from mature *Xenopus laevis* (9) (Nasco, Fort Atkinson, Wis.) and suspended in Barth solution [88 mM NaCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 5 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES; pH 7.6)]. Oocytes were dissociated, and follicle cells were removed by incubation in Ca^{2+}-free Barth medium containing 0.2% collagenase (type IA; Sigma Chemical Co., St. Louis, Mo.) for 120 min at room temperature. In some experiments, oocytes were manually dissected from their surrounding follicles (see below).

RNA was dissolved in distilled water at 0.2 to 0.4 mg/ml, and 10 to 50 nl was injected into the cytoplasm of stage VI oocytes (11). Typically, 300 to 600 oocytes were injected with each RNA preparation. Controls were injected with either brome mosaic virus (BMV) RNA (Promega Biotec, Milwaukee, Wis.) or distilled water. Uninjected oocytes gave the same results as did both of these controls. Oocytes were incubated in complete Barth solution at 18 to 22°C for 1 to 5 days.

2-Deoxy-D-glucose uptake. Oocytes (5 to 40 per group) were incubated for up to 120 min in 0.6 ml of Barth medium containing 10 μCi of 2-deoxy-D-[1,2-^{3}H]glucose (Amersham) and 2 mM 2-deoxy-D-glucose. Uptake was terminated by washing the oocytes with three 8-ml portions of ice-cold Barth medium containing 0.3 mM phloretin. The oocytes were dissolved in 1 ml of 2% sodium dodecyl sulfate (SDS), and the incorporated radioactivity was assayed by liquid scintillation counting. Control samples contained 50 μM cytochalasin B during the incubation. In studies of the time course of insulin action, oocytes were incubated first for 0 to 120 min in 0.3 ml of Barth medium containing 1 μM insulin (Eli Lilly & Co., Indianapolis, Ind.). A solution (0.3 ml) containing 10 μCi of 2-deoxy-D-[1,2-^{3}H]glucose and 4 mM 2-deoxy-D-glucose was then added, and uptake was measured. In studies of the concentration dependence of insulin or insulinlike growth factor I (IGF-I) action, the final concentration of ligand was varied from 1 pM to 10 μM. In assays of the reversibility of insulin action, oocytes were incubated in the presence of 1 μM insulin for 60 min, washed with insulin-free Barth medium, and incubated for 0 to 120 min before assays of 2-deoxy-D-glucose uptake.

Determination of RNA stability and Northern (RNA) blotting. Labeled RNA was injected into oocytes and recovered at 4, 24, 48, and 72 h after microinjection as described by Colman (8). After phenol-chloroform (1:1, vol/vol) extraction, nucleic acids were recovered by ethanol precipitation. The resulting precipitate was washed with 75% ethanol and suspended in distilled water containing RNase-free DNase (50 μg/ml; Pharmacia, Inc., Piscataway, N.J.). After 20 min at 37°C, the reaction mixture was extracted with phenol-chloroform; the RNA was recovered by two ethanol precipitations and quantitated by scintillation counting. For Northern blot analysis, the RNA was size fractionated by agarose gel electrophoresis (20 μg of RNA per lane) in the presence of formaldehyde and transferred to a Nytran membrane (Schleicher & Schuell, Inc., Keene, N.H.) (30). UV shadowing was used to assess the integrity and relative amounts of RNA loaded in each lane. Hybridizations were performed in solutions containing 50% formamide, 10% dextran sulfate, 2× Denhardt solution, 5× NaCl-citrate (0.75 M NaCl, 0.075 M trisodium citrate), and 1% SDS at 42°C. The blots were washed in 0.1% SDS–0.1× NaCl-citrate at 52°C. The probes used were either the full-length brain glucose transporter cDNA (4) or the full-length liver glucose transporter cDNA (31), each radiolabeled to a specific activity of 1×10^9 to 2×10^9 cpm/μg with [α-^{32}P]dCTP by the random-primer method (14).

Immunoprecipitation. Oocytes were metabolically labeled in Barth solution containing 20 μCi of [^{35}S]methionine per ml (>1,000 Ci/mmol; Amersham) for 24 h. Label was added 24 h after microinjection. Groups of 10 oocytes were solubilized in 0.5 ml of 0.8% SDS–1.0 M NaCl–5 mM EDTA–50 mM Tris hydrochloride (pH 7.8). One volume of 4% Triton X-100 was added, and the samples were incubated overnight at 4°C with a 1:50 dilution of the respective antisera. Immune complexes were adsorbed with protein A-Sepharose (Pharmacia), washed three times with 0.5 M NaCl–0.05% SDS–0.2% Triton X-100–2 mM EDTA–20 mM Tris hydrochloride (pH 6.8), and eluted in SDS-polyacrylamide gel electrophoresis sample buffer (27). After electrophoresis, immunoprecipitated proteins were visualized by fluorography.

Two of the antisera used in these studies have been previously characterized: the antiserum against the human erythrocyte glucose transporter (18) and the antiserum prepared against a synthetic peptide from the C-terminal region of the rat brain glucose transporter (19). For preparation of antisera against the liver glucose transporter, a synthetic peptide corresponding to amino acids 418 to 437 was synthesized on an automated Applied Biosystems peptide synthesizer and coupled to Affi-Gel 10 (Bio-Rad Laboratories, Richmond, Calif.) according to the recommendations of the supplier. The conjugates were emulsified with complete Freund adjuvant and injected subcutaneously into rabbits. Rabbits were boosted by subcutaneous injections of the antigen emulsified in incomplete Freund adjuvant, followed by a final intraperitoneal injection without adjuvant.

Isolation of oocyte plasma membranes. The oocytes were manually dissected from their surrounding follicles, and plasma membranes were isolated by manually opening oocytes with forceps (34). Cortical granules were removed as described by Wall and Patel (43).

Cytochalasin B binding. Cytochalasin B binding to intact oocytes was measured essentially as described by Gorga and Lienhard (16). Briefly, to 1 ml of Barth medium containing 40 to 80 stage VI oocytes, 5 μM cytochalasin E, and the desired concentration of sugar was added 10 μl of [^{3}H]cytochalasin B (5.0 to 10,000 μM cytochalasin B dissolved in absolute ethanol). Controls received 10 μl of absolute ethanol. After an incubation period that varied between 0 and 60 min, the total and free cytochalasin B concentrations were obtained by determining the radioactivity of duplicate 100-μl samples from the respective supernatants. The concentration of bound cytochalasin B was then calculated. In parallel experiments, cytochalasin B binding to oocytes was measured by counting the radioactivity retained on filters by using a rapid filtration procedure (16). The same results were obtained by both methods. To measure the binding of cytochalasin B to isolated plasma membranes, the samples were centrifuged at 4°C for 5 min in a microfuge, and two 100-μl samples of supernatant were assayed for radioactivity. In experiments in which sugar was added to displace or to block the binding of cytochalasin B to intact oocytes and to isolated plasma membranes, control samples were exposed to equivalent concentrations of L-glucose or sorbitol.

RESULTS

Stability and translation of microinjected RNAs encoding mammalian glucose transporters in *Xenopus* oocytes. In vitro transcription of the cDNAs encoding the glucose transporter

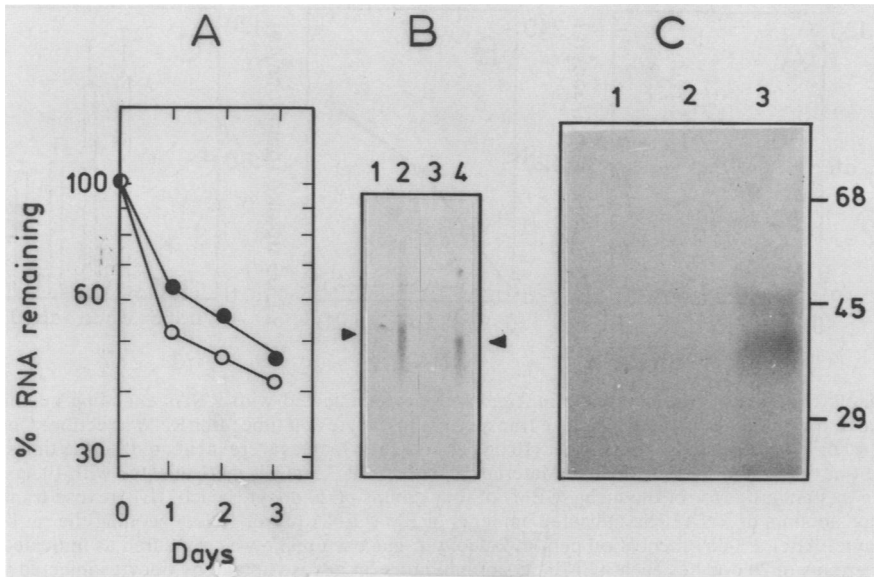

FIG. 1. Expression of mammalian glucose transport proteins in *Xenopus* oocytes. (A) Stability of microinjected RNAs. Oocytes were injected with high-specific-activity [α-^{32}P]RNAs (10 ng per oocyte) encoding the brain (○) or liver (●) glucose transporter. Groups of 30 oocytes were removed at the times indicated, and the amount of [^{32}P]RNA remaining was determined as described in Materials and Methods. (B) Blot hybridization analysis of glucose transporter-specific RNAs. Oocytes were injected with RNA encoding the rat brain or liver glucose transporter. Groups of 30 oocytes were removed 4 h after injection, and total RNA was isolated and fractionated (20 μg per lane) by electrophoresis on a formaldehyde–1% agarose gel. After blotting onto a nylon membrane, the blot was hybridized to brain (lanes 1 and 2) or liver (lanes 3 and 4) glucose transporter-specific cDNA labeled with ^{32}P. Lanes 1 and 3, RNA from uninjected oocytes; lane 2, RNA from oocytes injected with RNA encoding the brain glucose transporter; lane 4, RNA from oocytes injected with RNA encoding the liver glucose transporter. Arrowheads indicate positions of expected full-length transcripts. (C) Immunoprecipitation of the brain glucose transporter protein expressed in *Xenopus* oocytes. Oocytes were solubilized and subjected to immunoprecipitation, using preimmune serum (lane 1) or antipeptide antiserum against the C-terminal domain of rat brain glucose transporter (lanes 2 and 3). Lanes 1 and 3, Oocytes injected with brain glucose transporter-specific mRNA; lane 2, oocytes injected with water. Positions of molecular weight standards (in thousands) are shown on the right.

yielded homogeneous RNAs with transcript lengths of 2.5 kilobases (brain glucose transporter cDNA) and 2 kilobases (liver glucose transporter cDNA). Radioactive RNAs were synthesized to examine the stability of the microinjected transcripts. The amount of radioactivity recovered as ethanol-precipitable counts revealed that neither RNA was rapidly degraded after microinjection into *Xenopus* oocytes (Fig. 1A). Total recoverable ethanol-insoluble radioactivity decreased by 50% over the first 24 h postinjection, followed by a slower decrease between 24 and 72 h postinjection. Agarose gel electrophoresis followed by transfer to Nytran membranes revealed that approximately 40% of the remaining radioactivity was present as full-length transcripts as late as 72 h after microinjection. Hybridization to ^{32}P-labeled brain or liver glucose transporter cDNA, under conditions that gave a strong positive signal in cells injected with the appropriate synthetic RNA, failed to reveal a detectable signal in lanes that contained total RNA (20 μg) from uninjected oocytes (Fig. 1B).

To test whether the in vitro-synthesized RNAs are actively translated in *Xenopus* oocytes, injected and uninjected cells were incubated in the presence of [^{35}S]methionine, and detergent extracts were prepared and subjected to immunoprecipitation, using antibodies specific to each transporter. Polypeptides migrating in SDS-polyacrylamide gel electrophoresis with apparent molecular weights of approximately 40,000 were detected in oocytes injected with RNA encoding the brain glucose transporter protein (Fig. 1C). In addition, a less rapidly migrating band was detected in oocytes injected with RNA encoding the liver glucose transporter (not shown). As expected, similar proteins were not detected in control oocytes. Moreover, the antibodies directed against the human erythrocyte and the rat brain glucose transporter did not react with the protein recognized by the antibodies prepared against the rat liver glucose transporter. This result was predicted by differences in the primary structures of these proteins (4, 31, 41).

Expression of functional glucose transporters in *Xenopus* oocytes. Preliminary experiments with uninjected *Xenopus* oocytes revealed the presence of an endogenous glucose transport activity. In 20 experiments carried out with oocytes obtained from 14 animals, the initial rate of uptake of 2 mM 2-deoxy-D-glucose was 0.64 ± 0.12 pmol per oocyte per min. This uptake was completely inhibited by 0.1 mM cytochalasin B, an inhibitor of the mammalian facilitative glucose transporter.

When oocytes were injected with 10 ng of either the rat brain or liver glucose transporter RNA, the uptake of 2-deoxy-D-glucose was markedly increased (Fig. 2A). A significant increase in uptake was observed as early as 24 h after the injection, and the glucose uptake activity increased progressively for up to 4 to 5 days. These results are consistent with the long half-life of the injected RNAs. On the other hand, no increase in uptake was observed in oocytes injected with BMV RNA (Fig. 2A) or distilled water. Oocytes injected with various amounts of RNA encoding the brain glucose transport protein showed a dose-dependent increase in 2-deoxy-D-glucose uptake, an increase not apparent in control cells injected with different amounts of BMV RNA (Fig. 2B). These results demonstrate that oocytes of *X.*

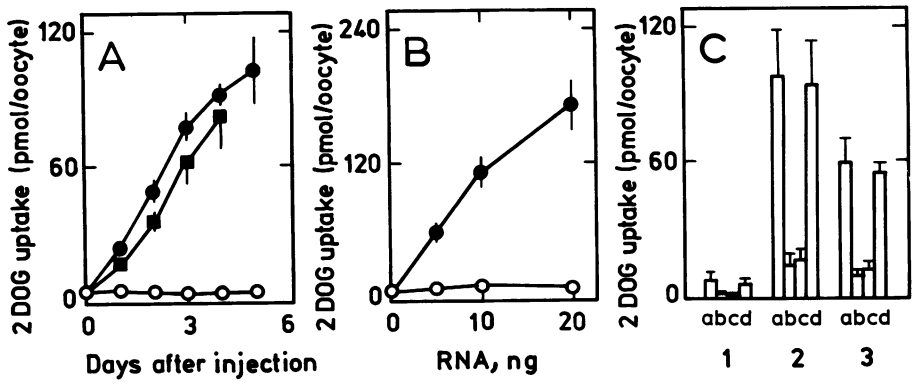

FIG. 2. Functional characterization of hexose uptake in *Xenopus* oocytes injected with RNAs encoding mammalian glucose transport protein. (A) Dependence of expression of functional glucose transporters in oocytes on time after RNA injection. Oocytes were injected with RNA encoding rat brain (●) or liver (■) glucose transporter (10 ng per oocyte). Oocytes were incubated for the times indicated, and 2-deoxy-D-glucose (2DOG) uptake was determined as described in Materials and Methods. Controls were injected with 10 ng of BMV RNA (○). Each point represents the mean ± standard error of the mean (SEM) of four groups of 20 oocytes each. (B) Hexose transport activity in oocytes microinjected with various amounts of RNA. The indicated amounts of BMV RNA (○) or RNA encoding the rat brain glucose transporter (●) were injected per oocyte. After a 3-day incubation period, 2-deoxy-D-glucose uptake was measured as indicated. Each point represents the mean ± SEM of four groups of 20 oocytes each. (C) Effects of inhibitors on hexose uptake by oocytes injected with water (bars 1), with 10 ng of RNA encoding the brain glucose transporter (bars 2), and with 10 ng of RNA encoding the liver glucose transporter (bars 3). Assays were performed 3 days after injection. 2-Deoxy-D-glucose uptake was assayed in the presence of 20 μM cytochalasin B (bars b), 250 mM D-glucose (bars c), or 250 mM L-glucose (bars d). Controls (bars a) were assayed as usual (see Materials and Methods). Data represent the mean ± SEM of three groups of 30 oocytes each.

laevis not only are able to translate microinjected exogenous RNAs encoding two different mammalian glucose transporters but also incorporate the synthesized proteins into their plasma membranes in a functionally active state.

This last point was further addressed by examining the response of the expressed transporters to different reagents that have been used to characterize the mammalian facilitative glucose transporter proteins. It was found that cytochalasin B, a powerful inhibitor of the facilitated glucose transport in mammalian cells (45), inhibited the uptake of 2-deoxy-D-glucose in oocytes injected with RNAs encoding the brain or liver glucose transporter (Fig. 2C). In addition, the uptake of 2-deoxy-D-glucose in these injected oocytes was sensitive to inhibition by elevated concentrations of D-glucose but not by equivalent concentrations of L-glucose. Thus, expression of these mammalian glucose transporters in *Xenopus* oocytes does not alter two of their most typical properties, stereospecificity and inhibition by cytochalasin B (45). As described above, the activity of the endogenous transporter expressed in uninjected oocytes was also inhibited by micromolar concentrations of cytochalasin B, and competition studies using D- and L-glucose indicated that this activity was also stereospecific (Fig. 1C). These results suggest that *X. laevis* oocytes express an endogenous transporter functionally related to the mammalian facilitative glucose transporters.

In the experiments analyzed above, the differences between the endogenous transport of oocytes and that expressed after the injection of the in vitro-synthesized RNAs were attributed to different levels of expression of the transporter proteins. We decided to determine whether we could detect differences in the kinetic parameters of 2-deoxy-D-glucose uptake mediated by the different transporters. Initial studies indicated that the uptake of 2-deoxy-D-glucose by injected and uninjected oocytes increased linearly with time for at least 10 min. As expected from results of the uptake experiments described above, uninjected oocytes could be clearly distinguished from those injected with the RNAs encoding rat brain and liver glucose transporters, not only by the maximum value of transport attained but also by the slope of the line of uptake versus time in the initial zone of linearity (data not shown). Lineweaver-Burk transformations of data from assays conducted for short periods of time using different concentrations of substrate were performed (Fig. 3). The results showed a large increase in the V_{max} for transport of 2-deoxy-D-glucose in injected oocytes compared with uninjected controls. The V_{max} values were 1.2, 25, and 20 pmol per oocyte per min for uninjected or control oocytes and for oocytes injected with RNA encoding the rat brain and liver glucose transporters, respectively. It is interesting that the endogenous oocyte

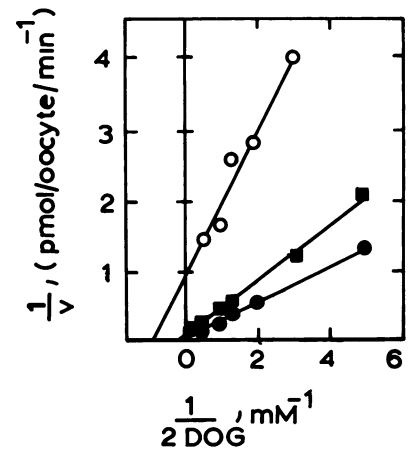

FIG. 3. Kinetic analysis of 2-deoxy-D-glucose uptake in injected and uninjected oocytes. 2-Deoxy-D-glucose (2DOG) uptake was measured for 5 min, using two groups of 20 to 80 oocytes per point. The results are presented in a Lineweaver-Burk plot. Assays were conducted 3 days after RNA injection (10 ng per oocyte). Symbols: (●) Oocytes injected with RNA encoding the brain glucose transporter; (■) oocytes injected with RNA encoding the liver glucose transporter; (○) uninjected oocytes.

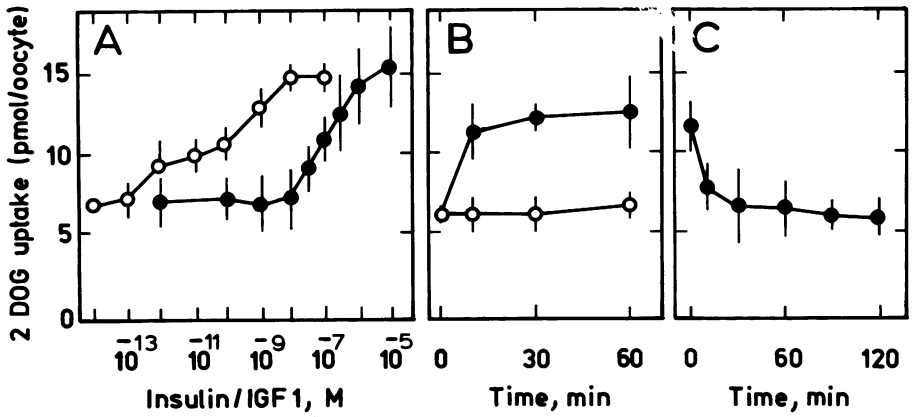

FIG. 4. Effect of insulin and IGF-I on hexose uptake by uninjected oocytes. (A) Dose-response curve for insulin and IGF-I. Oocytes were incubated in Barth medium containing 0.05% bovine serum albumin and the indicated concentrations of insulin (●) or IGF-I (○). After incubation for 60 min at 18°C, 2-deoxy-D-glucose (2DOG) uptake was assayed. Each point represents the mean ± SEM of three groups of 50 oocytes each. (B) Time course of insulin effect on hexose uptake. Oocytes were incubated at 18°C in Barth medium containing 0.05% bovine serum albumin and 1 μM insulin (●) for the indicated periods of time. 2-Deoxy-D-glucose uptake in the presence of 1 μM insulin was then assayed. Controls (○) were incubated without insulin. Data represent the mean ± SEM of three groups of 30 oocytes each. (C) Reversibility of insulin effect on hexose uptake. Oocytes were incubated in Barth medium containing 0.05% bovine serum albumin and 1 μM insulin for 60 min at 18°C. After washing, oocytes were incubated in insulin-free medium for the indicated periods of time, and 2-deoxy-D-glucose uptake was assayed in the absence of insulin. Data represent the mean ± SEM of three groups of 30 oocytes each.

transporter exhibited a K_m for 2-deoxy-D-glucose of 1.0 mM, significantly different from the K_ms calculated for rat brain (5 mM) and liver (7 mM) glucose transporters expressed in *Xenopus* oocytes. In the case of these last two transporters, the K_ms for 2-deoxy-D-glucose are similar to the values reported for the endogenously expressed mammalian proteins (28, 38).

Effect of insulin and IGF-I on 2-deoxy-D-glucose uptake. Since *Xenopus* oocytes are responsive to insulin (12, 13, 29, 35, 44), we decided to test whether insulin affected the activity of the transfected glucose transporters expressed by these cells. When uninjected oocytes were incubated in the presence of insulin, an increase in the uptake of 2-deoxy-D-glucose was observed (Fig. 4A). The effect was half-maximal at approximately 100 to 150 nM insulin and at 0.1 nM IGF-I. Exposure of oocytes to 1 μM insulin or 10 nM IGF-I increased the rate of uptake 1.8- to 2.2-fold. This result was consistently obtained with at least 15 different batches of oocytes obtained from 10 different frogs. We did not find any correlation between the insulin-induced maturation of the oocytes (12, 13, 29, 35, 44) and the increase in 2-deoxy-D-glucose uptake. In fact, of six batches of oocytes tested, only one underwent maturation in response to 1 μM insulin, but all showed a twofold increase in the uptake of 2-deoxy-D-glucose. The full stimulatory effect of insulin on 2-deoxy-D-glucose uptake was observed after 30 min of incubation with insulin (Fig. 4B). This stimulatory effect was rapidly and almost completely reversed over a 60-min period by incubation of the oocytes in insulin-free medium (Fig. 4C). This reversibility of the insulin effect is also typically observed in mammalian tissues and appears to be an essential characteristic of the mechanisms that determine the regulatory response to insulin (5, 10, 40, 45).

The stimulatory effect of insulin on the uptake of 2-deoxy-D-glucose was also observed in oocytes expressing the rat brain and liver glucose transporters (Fig. 5). In both

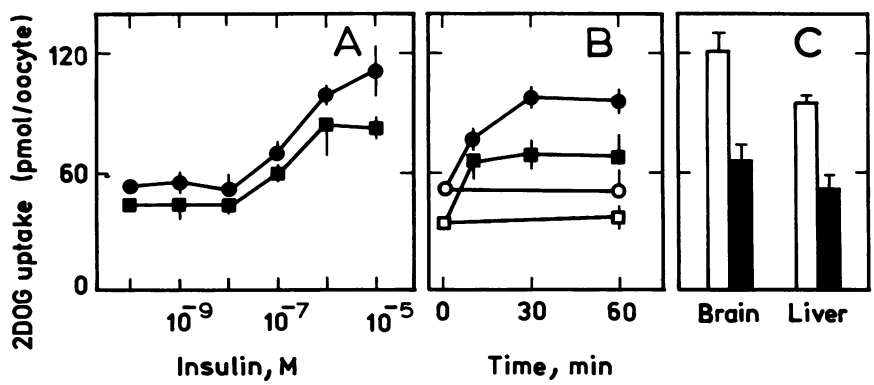

FIG. 5. Effect of insulin on hexose uptake by injected oocytes. Oocytes were injected with RNA encoding the brain (●) or liver (■) glucose transporter. Assays were conducted 3 days after RNA injection. (A) Dose-response curve of insulin effect on 2-deoxy-D-glucose (2DOG) uptake. Assays were conducted exactly as described for Fig. 4A. Data represent the mean ± SEM of four groups of 20 oocytes each. (B) Time course of insulin effect on hexose uptake. Oocytes were incubated in the presence (●, ■) or absence (○, □) of 1 μM insulin. For details, see the legend to Fig. 4B. Data represent the mean ± SEM of three groups of 20 oocytes each. (C) Reversibility of insulin effect on hexose uptake. Assays were conducted as described for Fig. 4C except that hexose uptake was measured after 0 (□) and 120 (■) min of preincubation with insulin-free medium. Data represent the mean ± SEM of four groups of 20 oocytes each.

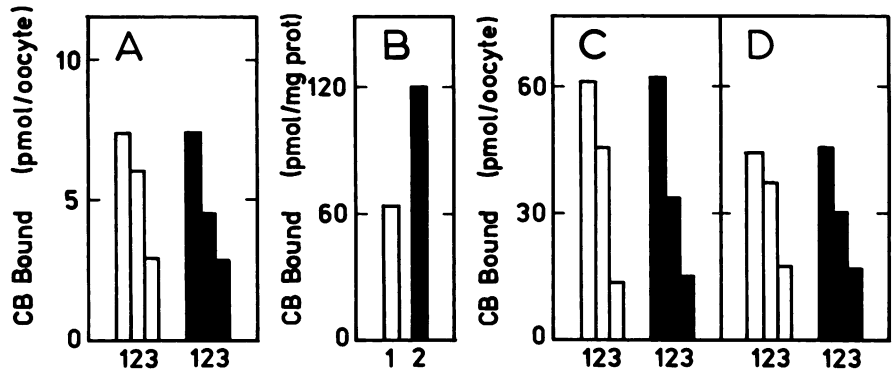

FIG. 6. Characterization of cytochalasin B-binding sites in oocytes. (A) Binding of cytochalasin B (CB; 250,000 cpm) to uninjected oocytes. Cytochalasin B (1 μM) binding to intact oocytes was measured in the presence of 250 mM L-glucose (bars 1), 250 mM D-maltose (bars 2), or 250 mM D-glucose (bars 3). This last group of oocytes was first incubated for 30 min with 250 mM D-glucose. In all three cases, oocytes were first incubated with (■) or without (□) 1 μM insulin for 60 min at 18°C. For experimental details, see Materials and Methods. Data represent the mean of two determinations, using 35 oocytes per experiment. Variation between the duplicates was approximately 5%. (B) Binding of cytochalasin B to purified plasma membranes. Plasma membranes were prepared from oocytes that were previously incubated for 60 min in the presence (bar 2) or absence (bar 1) of 1 μM insulin. Data represent the mean of two different experiments with membranes from 75 oocytes (0.7 mg of protein). For details, see Materials and Methods. Variation between the two experiments was less than 10%. (C) Binding of cytochalasin B to oocytes injected with RNA (10 ng per oocyte) encoding the brain glucose transporter. Assays were carried out 3 days after RNA injection. For details, see above (panel A) and Materials and Methods. (D) Binding of cytochalasin B to oocytes injected with RNA (10 ng per oocyte) encoding the liver glucose transporter. Binding was measured 3 days after RNA injection. For details see above (panel A).

cases, the response to insulin was similar to that observed for the endogenous oocyte transport, e.g., extent of activation as a function of insulin concentration (Fig. 5A), time course (Fig. 5B), and reversibility (Fig. 5C). The effect of insulin on the activity of the expressed transporters was consistently observed in at least six batches of oocytes, from different frogs, injected with four different preparations of the in vitro-synthesized RNAs.

Potential mechanism of activation of 2-deoxy-D-glucose uptake by insulin. The results presented above prompted us to determine whether the stimulatory effect of insulin was accompanied by the translocation of intracellular transporters to the plasma membrane, a phenomenon observed in mammalian cells exhibiting insulin-sensitive hexose transport (5, 10, 38, 40, 45).

Initial kinetic studies with uninjected oocytes indicated that 1 μM insulin elicited an increase in the V_{max} for transport of 2-deoxy-D-glucose (from 0.95 to 2.0 pmol per oocyte per min) but had no effect on K_m (1.2 mM without insulin; 1.3 mM with insulin). Although this result is consistent with translocation, it does not distinguish between translocation of intracellular transporters and activation of latent transporters present in the cell membrane (3, 5, 6, 10, 25, 38–40, 42, 45).

This point can be evaluated by measuring cytochalasin B binding. Under rigorously controlled experimental conditions, the number of cytochalasin B-binding sites correlates with the number of glucose transporters present in a given preparation (1–3, 6–8, 10, 17, 20, 23, 25, 33, 37, 40). In preliminary experiments, we found that binding of cytochalasin B to oocytes reached equilibrium within 1 min after its addition to oocytes. We next studied the effect of preincubating the oocytes for 5 min with different concentrations of cytochalasin B (from 10^{-4} to 10^{-8} M) on 2-deoxy-D-glucose uptake activity. Our results showed that cytochalasin B inhibited uptake of 2-deoxy-D-glucose into oocytes, with an apparent K_i of 2.3 μM. The same results were obtained with collagenase-treated and manually dissected oocytes. In both cases, 100 μM cytochalasin B inhibited uptake by over 95%. In other experiments, we measured the binding of cytochalasin B to intact oocytes. The specific binding to oocytes (binding in the presence of 500 mM L-glucose minus binding in the presence of 500 mM D-glucose) was saturable, with an apparent K_d of approximately 2 μM. Again, no differences were observed between oocytes isolated by collagenase treatment and those isolated by manual dissection. The observation that the K_d for the binding of cytochalasin B to intact oocytes is similar to its K_i for the inhibition of 2-deoxy-D-glucose uptake supports the notion that quantitation of the cytochalasin B-binding sites can be used to estimate the number of glucose transporters in our system (1–3, 6–8, 10, 17, 20, 23, 25, 33, 37, 40).

To measure the exposure of the cytochalasin B-binding sites to the extracellular medium, we examined the binding of cytochalasin B to intact oocytes in the presence and absence of maltose, a nontransported sugar known to be a strong inhibitor of the binding of cytochalasin B to the erythrocyte glucose transporter (2, 8). Maltose would be expected to inhibit binding of cytochalasin B to cell surface transporters but not to affect its binding to transporters that reside in intracellular membranes (7, 20, 33). No changes were observed in the total binding of cytochalasin B, in the presence of L-glucose or sorbitol, when oocytes were incubated for 60 min in the presence of 1 μM insulin as compared with controls incubated without insulin (Fig. 6A). Therefore, insulin did not produce an increase in the number of cytochalasin B-binding sites. In the presence of maltose, on the other hand, we observed a differential decrease in the binding of cytochalasin B to those oocytes that were incubated with insulin (37% inhibition) versus the controls without previous treatment (18% inhibition) (Fig. 6A). In control experiments, oocytes were incubated in the presence of D-glucose for 30 min before addition of cytochalasin B to the medium in order to load the cells with glucose. Under these conditions, one would expect there to be inhibition of the binding of cytochalasin B to the glucose transporters in both subcellular locations (20, 33, 42, 44). In confirmation of this expectation, we observed a 60% inhibition in the binding of cytochalasin B to the oocytes as compared with controls incubated with L-glucose. This value was not affected by

previous exposure of the oocytes to insulin (Fig. 6A). Taken together, these data support the notion that in *Xenopus* oocytes, the action of insulin is associated with the translocation of glucose transporters to the plasma membrane. To further confirm this conclusion, we measured the binding of cytochalasin B to purified plasma membranes prepared from oocytes previously incubated in the presence or absence of insulin. Plasma membranes purified from oocytes treated with insulin exhibited an increased specific binding of cytochalasin B (D-glucose-inhibitable cytochalasin B binding) compared with membranes purified from oocytes preincubated without insulin (Fig. 6B).

We then measured the binding of cytochalasin B to oocytes expressing the rat brain and liver glucose transporters. As expected, these oocytes showed a clear increase in the total binding of cytochalasin B when compared with uninjected oocytes (Fig. 6C and D). In both cases, maltose-induced inhibition of cytochalasin B binding was more marked in insulin-treated oocytes (46 and 33% inhibition in oocytes expressing the rat brain and liver glucose transporters, respectively) than in the controls (25 and 16% inhibition in oocytes expressing the rat brain and liver glucose transporters, respectively). This result suggests that an increased number of glucose transporters is expressed on the oocyte surface after treatment with insulin.

DISCUSSION

Our results demonstrate that the injection of in vitro-synthesized RNAs encoding either the rat brain or liver glucose transporter results in the expression of functional facilitative hexose transporters in the oocyte plasma membrane. Uninjected *Xenopus* oocytes exhibit endogenous hexose transport activity. Our data suggest that this activity corresponds to the expression of a transporter whose functional characteristics are similar to those of the mammalian facilitative glucose transporters. The K_m of the endogenous oocyte transporter for 2-deoxy-D-glucose was, however, significantly lower than the K_ms of the rat brain and liver transporters. The results of the immunoprecipitation analyses, together with the RNA blots, suggest that this functional difference between the *Xenopus* and mammalian transporters may reflect differences in the primary structures of the transporters.

Both microinjected transporter RNAs appear to be quite stable. This finding correlates with the observation that the appearance of functional glucose transporters on the surface of *Xenopus* oocytes increased for at least 4 to 5 days after microinjection. When uptake of 2-deoxy-D-glucose was measured 3 days after the microinjection of the RNAs, groups of oocytes taken from different frogs expressed glucose transport at comparable levels. Consistently, microinjected oocytes transported 2-deoxy-D-glucose at a velocity 15- to 20-fold greater than did uninjected controls. As expected, the expressed transporters were recognized by specific antibodies directed against them, and their functional properties were similar to those described for the transporters expressed in mammalian cells and tissues (38, 45, 46). An observation made in the course of these studies is that insulin stimulates the activity of the endogenous oocyte transporter, independent of its effects on oocyte maturation (12, 13, 29, 35, 44). Although insulin stimulated the uptake of 2-deoxy-D-glucose only twofold, the effect was consistently observed in oocytes prepared from different frogs. The characteristics of the time course of the insulin effect and its reversibility are similar to those observed in mammalian tissues that respond to insulin (5, 10, 25, 38, 40, 45). However, the concentration of insulin needed to half-maximally activate the endogenous oocyte glucose transporters (150 nM) is at least 100-fold greater than the concentration reported for half-maximal activation of the rat adipocyte glucose transporter (5, 10, 40). Previous descriptions of insulin-induced maturation of *Xenopus* oocytes have indicated that insulin concentrations of this kind are needed to induce any response of the cells to insulin (12, 13, 29, 35, 44). In addition, it has been reported that the effect of insulin on oocyte maturation is mediated through an IGF-I receptor rather than an insulin receptor (29, 35). Our data are consistent with this suggestion, since oocyte transport is, in fact, more sensitive to IGF-I than to insulin (Fig. 4A). This enhanced sensitivity is also observed with the microinjected oocytes expressing the mammalian transporters. Thus, the requirement for high concentrations of insulin to induce activation of the glucose transporters appears to be a rather general property of the oocyte and not necessarily specific for the glucose transporter. The likelihood that insulin promotes translocation of the transporter in oocytes provides additional evidence that glucose transport in oocytes is similar to that in mammalian cells.

It is interesting that both mammalian glucose transporters expressed in *Xenopus* oocytes are insulin sensitive. Moreover, the characteristics of their responses to insulin match those observed for endogenous oocyte transport, including half-maximal activation by 100 to 150 nM insulin and probable translocation of transporters to the plasma membrane. Translocation in response to insulin has been observed in certain insulin-responsive cells, such as adipocytes and muscle cells (3, 5, 6, 10, 25, 38–40, 42, 45), but not in brain and liver cells (1, 5, 10, 16, 38, 40, 45). As far as is known, brain and liver hexose transport is not sensitive to stimulation by insulin (38, 45). Thus, the insulin sensitivity of a glucose transporter may be determined, at least in part, by the cell type in which the transporter is being expressed. This conjecture suggests the possibility that there are certain tissue- or cell-specific factors involved in regulating the activity of transport. This conclusion is consistent with recent reports that both the HepG2 and rabbit brain glucose transporters respond to insulin when expressed in mammalian cells containing endogenous insulin-sensitive glucose transporters (1, 16).

Although mammalian glucose transporters have been expressed in bacteria (36, 41), our results highlight the advantages of using oocytes of *X. laevis* as an expression system with which to dissect the structural features required for hexose transport and the potential role of additional cell-specific factors in the regulation of this process.

As this report was being prepared, an article by Janicot and Lane was published that summarized their results with the endogenous *Xenopus* oocyte glucose uptake system (22). The results they published for K_m of 2-deoxy-D-glucose uptake, insulin and IGF-I sensitivity, and inhibition by cytochalasin B are similar to those reported here. They, as we, found that the kinetics, insulin sensitivity, and inhibition by cytochalasin B were similar for 3-*O*-methylglucose uptake and 2-deoxy-D-glucose uptake. We both suggest that the effect of insulin may be mediated by oocyte IGF-I receptors.

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