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# Activation of glucose uptake by insulin and insulin-like growth factor I in *Xenopus* oocytes

(tyrosine kinase/insulin-like growth factor I receptor/insulin receptor)

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ABSTRACT **_Xenopus laevis_ oocytes possess a glucose transport system that is activated 3- to 5-fold by insulin-like growth factor I ($K_a$ = 3 nM) and insulin ($K_a$ = 200–250 nM), properties suggesting activation mediated by an insulin-like growth factor I receptor. This activation increases the $V_{max}$ of hexose uptake and has little or no effect on the $K_m$ for deoxyglucose ($K_m$ = 1–2 mM). Activation by hormone requires about 60 min and is inhibited by cytochalasin B but not by cycloheximide. The dependence of hexose uptake rate on hexose concentration exhibits cooperativity with Hill coefficients of 1.8 and 1.4 for the basal and hormone-activated states, respectively. Microinjection of a monoclonal antibody directed against the tyrosine kinase domain of the human insulin receptor blocks activation of hexose uptake by insulin-like growth factor I and insulin but has no effect on basal uptake. Taken together the results implicate the tyrosine-specific protein kinase activity of a cell-surface insulin-like growth factor I receptor in the activation of glucose transport in the *Xenopus* oocyte.**

The activation of glucose uptake by insulin occurs in only a few cell types, notably skeletal and heart muscle cells and adipocytes (1). Although the intervening steps in signal transmission from the cell-surface insulin receptor to the glucose transporter have not yet been characterized, several important facts have emerged. It has been established that binding of insulin to its receptor activates the intrinsic tyrosine-specific protein kinase within the cytoplasmic domain of the receptor (2). There is now ample evidence to implicate the receptor kinase in signal transmission to known end targets of insulin action, including the glucose transport system (3). It is also known (4, 5) that upon stimulation of adipocytes or muscle cells with insulin, the glucose transporter undergoes translocation from an intracellular storage site to the plasma membrane, thereby causing increased glucose uptake. These characteristics of the insulin-stimulated glucose transport system make investigation of the activation mechanism difficult in typical animal cells or in a cell-free system.

A promising alternative cell-type with which to study the mechanism of insulin-stimulated glucose uptake would be the *Xenopus* oocyte. The large size of this cell makes it possible to microinject specific reagents that interrupt or activate signal transmission to the glucose transporter (or other end targets of insulin action) at points in the pathway beyond the cell-surface receptor. Baulieu and coworkers (6) have reported that insulin can mimic progesterone by inducing cell division in oocytes and that the response to insulin is most likely mediated through the insulin receptor. Insulin (but not progesterone) action on oocyte maturation is blocked by an anti-insulin receptor antibody directed against the tyrosine kinase domain of the receptor's β-subunit (7). Other activities have also been shown to be affected by insulin or insulin-like growth factor I (IGF-I) in *Xenopus* oocytes, including cAMP phosphodiesterase and adenylate cyclase (8), phosphorylation of ribosomal protein S6 (9, 10), membrane alkaline phosphatase (11), and membrane serine kinase (12).

In this communication, we report that *Xenopus laevis* oocytes possess a glucose transport system that is markedly activated by physiological concentrations of IGF-I and by nonphysiologically high levels of insulin. These results, taken together with those from microinjection experiments with an anti-tyrosine kinase domain antibody, suggest that both hormones exert their activating effects on hexose uptake through the tyrosine kinase of an endogenous IGF-I receptor. Thus, the *Xenopus* oocyte should be useful in defining the steps in signal transmission between the receptor kinase and the glucose transport system.

## MATERIALS AND METHODS

**Materials.** Bovine serum albumin (RIA grade), 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, cytochalasin B, and phloretin were from Sigma. 2-Deoxy-D-[U-$^{14}$C]glucose and 3-*O*-methyl-D-[1-$^{14}$C]glucose were from New England Nuclear. 17A3 monoclonal antibody (7) and a nonrelated mouse IgG were generously provided by Richard Roth (Stanford University). Insulin and IGF-I were provided by Eli Lilly.

**Oocyte Isolation.** Adult *X. laevis* females were obtained from Nasco (Fort Atkinson, WI) and maintained as described by others (13). Several lobes of ovary were surgically removed by laparotomy from dormant females, not treated with human chorionic gonadotropin, that had been anesthetized by hypothermia (30 min in ice water). The lobes were rinsed with modified Barth's solution [(MBS): 0.82 mM $MgSO_4$/0.41 mM $CaCl_2$/1 mM KCl/0.33 mM $Ca(NO_3)_2$/88 mM NaCl/2.4 mM $NaHCO_3$/10 mM Hepes buffer, pH 7.6], after which stage 5 and 6 oocytes were manually dissected. Following incubation for 2 hr at 18°C in MBS, healthy oocytes were selected and maintained (10 oocytes per ml of MBS) in 10-cm cell culture dishes at 18°C with a medium change every 12 hr.

**Oocyte Maturation.** Twenty to 30 oocytes in 3 ml of MBS containing 0.1% bovine serum albumin and in the absence or presence of the appropriate hormone were incubated in 3.5-cm culture dishes for 18 hr at 18°C. Oocytes were examined microscopically for maturation by the appearance of a "white spot" in the pigmented animal pole, which is an indicator of germinal vesicle breakdown (GVBD) (14).

**Hexose Uptake Rate.** Ten oocytes were incubated for 90 min at 18°C in a 5-ml glass vial in 1 ml of MBS in the presence or absence of the appropriate hormone. 2-Deoxy-D-[U-$^{14}$C]-glucose (0.5 mM; 0.6 mCi/mmol; 1 Ci = 37 GBq) or 3-*O*-methyl-D-[1-$^{14}$C]glucose (0.25 mM; 1 mCi/mmol) was then

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Abbreviations: IGF-I, insulin-like growth factor I; MBS, modified Barth's solution; GVBD, germinal vesicle breakdown.

added and incubation at 18°C was continued for an additional 10 min. Hexose uptake was terminated by three rapid washes with 3 ml of ice-cold MBS, after which oocytes were lysed in 1 ml of 2.5% sodium dodecyl sulfate and cell-associated radioactivity was determined. Addition of cytochalasin B or phloretin in the quenching buffer had no effect on the results. After 60 min of hormone activation, hexose uptake rate remained constant for at least 2 hr.

**Microinjection of Oocytes.** Stage 5 and 6 oocytes (1.3–1.5 mm in diameter) were microinjected with 50–100 nl per oocyte of a solution containing 10–20 ng of the appropriate immunoglobulin per oocyte. Injected oocytes were incubated for 2 hr at 18°C in MBS, after which healthy oocytes were analyzed for hexose uptake.

## RESULTS AND DISCUSSION

In view of the advantages of the amphibian oocyte system for the analysis of complex cellular signal-transmission mechanisms (see Introduction), we set out to determine whether the *Xenopus* oocyte possesses an insulin- or IGF-I-responsive glucose uptake system. As shown in Fig. 1*A*, both insulin and IGF-I stimulated 2-deoxyglucose uptake 3- to 4-fold (up to 5-fold in several experiments) in a hormone concentration-dependent manner. Likewise, both peptide hormones induced oocyte maturation as measured by GVBD (Fig. 1*B*), a known response of the *Xenopus* oocyte to several hormones, including progesterone,* insulin, and IGF-I (8, 15). Though all three hormones induce GVBD, progesterone has no effect on 2-deoxyglucose uptake (results not shown).

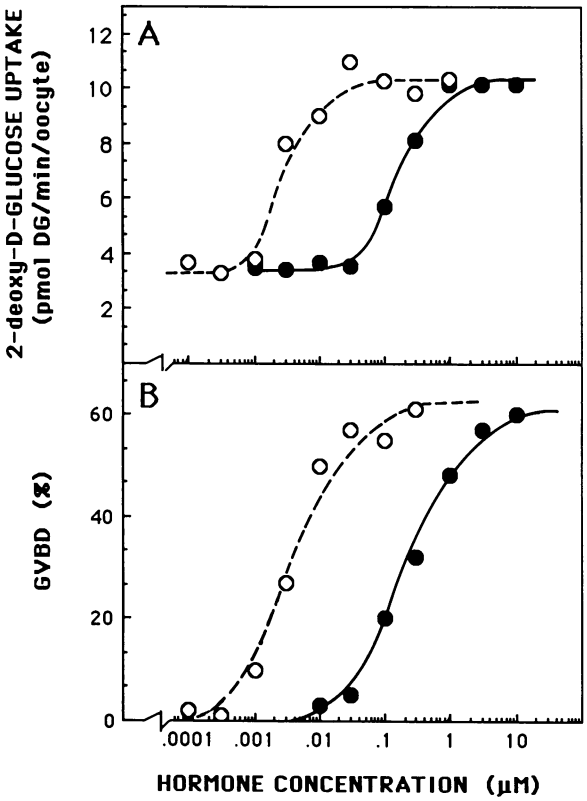


FIG. 1. Dose–response curves for insulin and IGF-I effects on 2-deoxyglucose (DG) uptake and GVBD. (*A*) Groups of 10 oocytes (in triplicate) were transferred into 1 ml of MBS containing 0.1% bovine serum albumin and the indicated micromolar concentrations of insulin (●) or IGF-I (○). After incubation for 90 min at 18°C, 2-deoxy-D-[U-$^{14}$C]glucose was added and further incubated for 10 min. Glucose uptake was then determined. Basal glucose uptake (without hormone addition) was 3.0 pmol of hexose uptake per min per oocyte. Similar results were obtained in two other experiments. (*B*) Groups of 20–35 oocytes (in triplicate) were transferred into 3 ml of MBS containing 0.1% bovine serum albumin and the indicated micromolar concentrations of insulin (●) or IGF-I (○). After overnight incubation at 18°C, oocytes were examined for the maturation response by the appearance of a white spot in the pigmented pole characterizing GVBD. The results are the mean of three experiments with oocytes from different frogs. Standard error values were <20%. Without hormone, GVBD was <2%.

Insulin and IGF-I are not equipotent either in activating hexose uptake or in inducing GVBD, the half-maximal effects for the activation of both processes occurring at 200–250 nM insulin or 3–3.5 nM IGF-I (Fig. 1). In contrast, with mouse 3T3-L1 adipocytes, which possess both insulin and IGF-I receptors, insulin and IGF-I activate hexose uptake 15-fold and exhibit virtually identical $K_a$ values of 3–8 nM (results not shown; see also ref. 16). The nearly 70-fold higher $K_a$ value of insulin than IGF-I for the activation of hexose uptake by the *Xenopus* oocyte strongly suggests that insulin exerts its effect in this system through the IGF-I receptor.† Insulin is known to bind to and elicit biological responses through the IGF-I receptor, but 50- to 100-fold higher concentrations of insulin than of IGF-I are required (17).

To verify that the hexose uptake system of *Xenopus* oocytes carries out facilitated glucose transport, the uptake of 3-*O*-methylglucose, a structural analogue of glucose, was tested in oocytes. Unlike 2-deoxyglucose, 3-*O*-methylglucose does not undergo phosphorylation by hexokinase (or glucokinase) after being taken up by cells; hence the uptake of this sugar more accurately reflects true facilitated transport (1). In experiments not shown it was demonstrated that 3-*O*-methyl-D-[1-$^{14}$C]glucose uptake occurs at a rate similar to that of 2-deoxyglucose and is activated by insulin and IGF-I to the same extent as 2-deoxyglucose uptake. Finally, as illustrated in Table 1 (experiment 1), both basal and hormone-activated 2-deoxyglucose uptake are drastically inhibited by cytochalasin B, a classical inhibitor of glucose transport in animal cells.

The kinetics of activation of 2-deoxyglucose uptake by saturating concentrations of insulin and IGF-I (1 μM and 30 nM, respectively) were measured in *Xenopus* oocytes maintained at 18°C (Fig. 2). Following hormone addition there was an unexpectedly long lag of 20–30 min, at which time deoxyglucose uptake rate increased abruptly, reaching a maximum at 60 min and then remaining constant for several hours. For this reason, all hexose uptake measurements were made after a 90-min preincubation with hormone, a time at which a constant maximal extent of activation has been achieved. The rate of activation of hexose uptake by insulin or IGF-I in the *Xenopus* oocyte is much slower than in adipocytes or muscle cells of mammals. In mouse 3T3-L1 adipocytes, for example, maximal hexose uptake rate is achieved within 3–5 min after hormone addition (results not shown; see also refs. 16 and 18). To ascertain whether the slow rate of activation of 2-deoxyglucose uptake by hormone in the *Xenopus* oocyte requires protein synthesis, experiments were performed in the presence (and absence) of 15 μM cycloheximide. Although cycloheximide inhibited protein synthesis by >95% (results not shown), the inhibitor had no effect on the activation of 2-deoxyglucose uptake by insulin or IGF-I (Table 1, experiment 2).

*The extent of GVBD induced by progesterone was 95% (results not shown).

†We recently demonstrated (results not shown) that *Xenopus* oocytes contain an IGF-I receptor that can be extracted from membranes with detergent and exhibits IGF-I-activated autophosphorylation and model substrate (reduced and carboxyamidomethylated lysozyme) phosphorylation. The $K_a$ values for both processes activated by IGF-I and insulin were 3 nM and 200–300 nM, respectively, consistent with the presence of an IGF-I receptor but not a detectable level of an insulin receptor in *Xenopus* oocytes.

Table 1. Effect of inhibitors on 2-deoxyglucose uptake

| Treatment | 2-Deoxy-D-[1-$^{14}$C]glucose uptake, pmol/min per oocyte | | |
|---|---|---|---|
| | Control | Insulin | IGF-I |
| Experiment 1 | | | |
| None | 3.2 ± 0.51 | 9.5 ± 0.72 | 8.3 ± 0.64 |
| Cytochalasin B | 0.51 ± 0.34 | 0.28 ± 0.08 | 0.87 ± 0.30 |
| Experiment 2 | | | |
| None | 3.3 ± 0.78 | 10.7 ± 1.9 | — |
| Cycloheximide | 3.8 ± 0.29 | 11.1 ± 1.2 | — |

Groups of 10 oocytes (in triplicate) were transferred into 1 ml of MBS in the presence or absence of either 1 μM insulin or 30 nM IGF-I. After incubation for 90 min at 18°C, 2-deoxy-D-[U-$^{14}$C]glucose was added and deoxyglucose uptake was measured for 10 min. Experiment 1: Cytochalasin B at a final concentration of 20 μM was added 10 min before the addition of labeled deoxyglucose. Experiment 2: Cycloheximide at a final concentration of 15 μM was added 30 min before addition of insulin. Results are expressed as the mean ± SEM of two or three experiments with oocytes from different frogs.

To determine whether the 3- to 4-fold activation of hexose uptake by insulin or IGF-I (Fig. 1A) results from a change in the $V_{max}$ or $K_m$ for hexose, the dependence of sugar uptake rate upon 2-deoxyglucose concentration was investigated. In this study, measurements of initial rates of hexose uptake were made only after steady-state rates of hormone-activated hexose uptake had been achieved—i.e., 90 min after hormone addition.‡ Thus, the hexose concentration dependence (or the effect of hormone on hexose concentration dependence) of 2-deoxyglucose uptake rate should reflect the intrinsic activity of cell-surface glucose transporters and not the translocation of glucose transporters from an intracellular compartment. As illustrated in Fig. 3A, uptake rate exhibits both saturation and sigmoidicity with respect to hexose concentration. Though not visualized optimally because of sigmoidicity of the plot (Fig. 3A), it is nevertheless evident that insulin has little, if any, effect on the apparent $K_m$ for 2-deoxyglucose but markedly increases $V_{max}$. An approximately 3-fold effect of insulin on $V_{max}$ is evident both in curvilinear Lineweaver–Burk plots (Fig. 3B) and in linearized plots of $1/v$ versus $1/S^2$ (Fig. 3C; see ref. 19). Taken together, these results indicate that insulin and IGF-I act primarily by increasing the $V_{max}$ of sugar uptake and have little or no effect on the apparent $K_m$ (1–2 mM) for hexose.

Lineweaver–Burk analysis emphasizes the apparent cooperativity of insulin-activated 2-deoxyglucose uptake with respect to hexose concentration, which is most pronounced in oocytes not stimulated by insulin (Fig. 3B). The extent of cooperativity analyzed as shown in Fig. 3D gave rise to Hill coefficients of 1.8 and 1.4 for the basal and hormone-stimulated cases, respectively. These findings indicate that hormone stimulation reduces cooperativity. The cooperative behavior of the glucose transport system and its alteration by hormone suggests that the cell-surface transporter can exist in different conformational states with differing "intrinsic activities." This phenomenon could have physiological significance and may be related to other recently described alterations in the intrinsic activity of cell-surface glucose transporters (20). Though the generality of the cooperative response of the glucose transporter observed with the oocyte system is unknown, the phenomenon bears further study. This property of the glucose transporter may have gone unnoticed in other cell systems because of the higher temperatures (often 37°C) generally used to study glucose uptake. The present investigation with *Xenopus* oocytes was conducted at 18°C to preserve oocyte viability, which deteriorates rapidly at higher temperatures (e.g., at 37°C).

‡Since a constant initial rate of hexose uptake is achieved within 60 min (Fig. 2), the number of glucose transporters at the cell surface should have reached a steady-state level within 90 min after hormone-induced translocation.

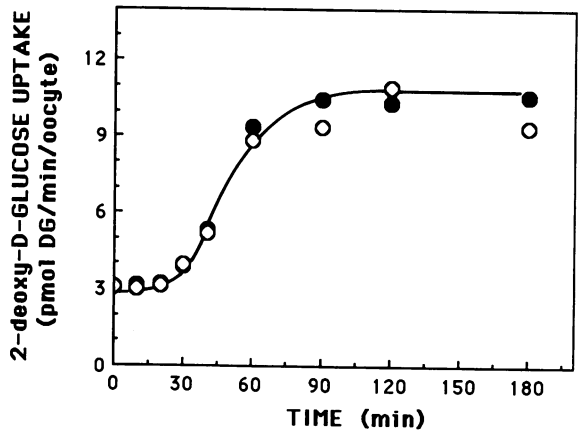


FIG. 2. Kinetics of insulin- and IGF-I-stimulated 2-deoxyglucose (DG) uptake rate. Groups of 10 oocytes (in triplicate) were transferred into 1 ml of MBS and incubated at 18°C in the presence of either 1 μM insulin (●) or 30 nM IGF-I (○) for the periods of time indicated. 2-Deoxy-D-[U-$^{14}$C]glucose uptake rates were then determined. Similar results were obtained in two other experiments.

Evidence implicating the cytoplasmic tyrosine kinase of the IGF-I receptor in the activation of hexose uptake in *Xenopus* oocytes was obtained by microinjection of a mouse monoclonal antibody directed against the tyrosine kinase domain of the human insulin receptor. This antibody, i.e. 17A3, was shown previously (7) to block GVBD in oocytes stimulated by extremely high levels of insulin. Though not reported, it is likely that this antibody also recognizes the tyrosine kinase domain of the IGF-I receptor, which has a high degree of amino acid sequence identity within this domain (21). As shown in Table 2, microinjection of monoclonal antibody 17A3 into *Xenopus* oocytes had no effect on basal hexose uptake but completely inhibited the activation of 2-deoxyglucose uptake by 30 nM IGF-I and 1 μM insulin. Microinjection of control mouse IgG had no effect on hormone-activated hexose uptake. Since hexose uptake activated by 30 nM IGF-I (a concentration at which activation by insulin is not observed) is inhibited by the anti-tyrosine kinase antibody, it appears that the IGF-I receptor, rather than the insulin receptor, mediates hormone-activated hex-

Table 2. Effect of microinjection of antireceptor tyrosine kinase antibody on 2-deoxyglucose uptake

| Treatment | 2-Deoxy-D-[U-$^{14}$C]glucose uptake, pmol/min per oocyte | | |
|---|---|---|---|
| | Control | Insulin | IGF-I |
| Control | 2.9 ± 0.53 | 8.7 ± 0.69 | 8.0 ± 0.51 |
| Microinjection | | | |
| Buffer | 3.0 ± 0.24 | 9.3 ± 0.55 | 8.8 ± 0.66 |
| Mouse IgG | 3.2 ± 0.52 | 9.0 ± 0.92 | 8.0 ± 0.75 |
| 17A3 IgG | 2.8 ± 0.54 | 3.2 ± 0.50 | 3.4 ± 0.40 |

Control and injected oocytes were placed in MBS and incubated for 2 hr at 18°C. Healthy oocytes, in groups of 10 (in triplicate), were then transferred into 1 ml of MBS in the presence or absence of either 1 μM insulin or 30 nM IGF-I. After incubation for 90 min at 18°C, labeled deoxyglucose was added and uptake was measured for 10 min. Where indicated, 10–20 ng of a mouse monoclonal antibody (17A3) against human insulin receptor tyrosine kinase (7) or 10–20 ng of an unrelated mouse IgG was microinjected per oocyte. The final concentration of antibody in the oocyte was calculated to be about 50 nM by assuming an intracellular volume of 1 μl per oocyte. The buffer for microinjection was composed of 83 mM NaCl/1 mM KCl/5 mM Tris·HCl, pH 7.4. Results are expressed as the mean ± SD of two experiments with oocytes from different frogs.

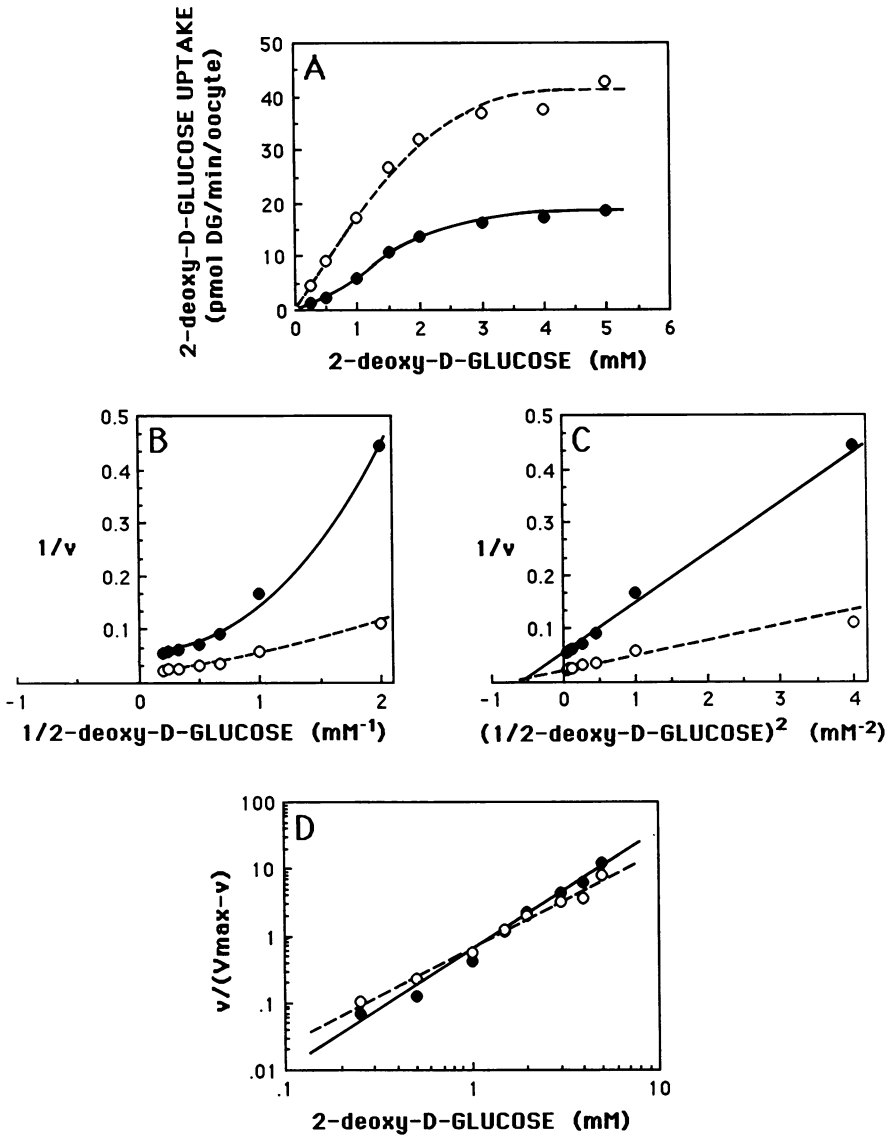


FIG. 3. Dependence of 2-deoxyglucose (DG) uptake rate on hexose concentration. (*A*) Groups of 10 oocytes (in triplicate) were transferred into 1 ml of MBS in the presence (○) or absence (●) of 1 $\mu$M insulin. After incubation for 90 min at 18°C, 2-deoxy-D-[U-$^{14}$C]glucose at the indicated final concentrations was added and hexose uptake rate was measured for 10 min. The results are representative of three different experiments. The results presented in *A* were replotted in *B*, Lineweaver–Burk form; in *C*, as $1/v$ versus $1/S^2$; and in *D*, Hill plot form. Hill coefficients calculated from the slopes of $\log(v/V_{max} - v)/\log S$ for basal and insulin-stimulated deoxyglucose uptake were 1.8 and 1.4, respectively; the calculated $K_m$ values were both about 1.4 mM.

ose uptake in the oocyte. Insulin at a concentration of 30 nM would have fully occupied the binding sites of typical insulin receptors had they been present in the oocyte. The fact that extremely high concentrations of insulin (i.e., about 1 $\mu$M) are required to fully activate hexose uptake in the oocyte (Fig. 1*A*) strongly suggests that insulin acts through the IGF-I receptor and that no, or very few, functional insulin receptors are present in this cell type.

The *Xenopus* oocyte possesses the necessary characteristics for reconstitution of insulin-stimulated glucose uptake. The oocyte lacks detectable levels of a functional insulin receptor but has a glucose transport system that is markedly stimulated by means of its IGF-I receptor tyrosine kinase (Table 2). Thus, by expressing the appropriate heterologous mRNAs (for the insulin receptor, insulin-responsive glucose transporter, and possibly other components of the insulin-stimulatable glucose uptake system) in the *Xenopus* oocyte, it should be possible to reconstitute a glucose uptake system, which is responsive to physiological concentrations of insulin, for mechanistic and molecular genetic studies.

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