ELSEVIER

Biochimica et Biophysica Acta 1419 (1999) 289–298

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.elsevier.com/locate/bba

Multiple effects of mercuric chloride on hexose transport in *Xenopus* oocytes

David M. Barnes [a,*], Destiny B. Sykes [b], David S. Miller [b]

[a] *Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR 72701, USA*
[b] *Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, USA*

Received 26 February 1999; received in revised form 4 May 1999; accepted 7 May 1999

Abstract

$HgCl_2$ had both stimulatory and inhibitory effects on [^{3}H]2-deoxyglucose (DG) uptake in *Xenopus laevis* oocytes. The Hg dose response was complex, with 0.1–10 μM Hg increasing total DG uptake, 30–50 μM Hg inhibiting, and concentrations >100 μM increasing uptake. Analyses of the effects of Hg on DG transport kinetics and cell membrane permeability indicated that low concentrations of Hg stimulated mediated uptake, intermediate concentrations inhibited mediated uptake, but high Hg concentrations increased non-mediated uptake. 10 μM Hg increased the apparent V_{max} for DG uptake, but caused little or no change in apparent K_m. Phenylarsine oxide prevented the increase in DG uptake by 10 μM Hg, suggesting that the increase was due to transporter recruitment. Microinjecting low doses of $HgCl_2$ into the cell increased mediated DG uptake. Higher intracellular doses of Hg increased both mediated and non-mediated DG uptake. Both insulin and Hg cause cell swelling in isotonic media and, for insulin, this swelling has been linked to the mechanism of hormone action. Osmotically swelling *Xenopus* oocytes stimulated DG transport 2–5-fold and this increase was due to an increased apparent V_{max}. Exposing cells to 10 μM Hg or 140 nM insulin both increased cellular water content by 18% and increased hexose transport 2–4-fold. These data indicate that low concentrations of Hg and insulin affect hexose transport in a similar manner and that for both an increase cellular water content could be an early event in signaling the increase in hexose transport.

Keywords: Mercury; Insulin; Hexose transport; Cell volume regulation; *Xenopus*

1. Introduction

Heavy metals such as mercury are potent cytotoxins. They can disrupt nearly every cell function, including membrane transport, volume regulation, protein and RNA synthesis and signal transduction [1]. Although these effects on transport and metabolism are generally inhibitory, low concentrations of mercurials have been shown to stimulate hexose transport in adipocytes and protein synthesis in both liver [2] and brain [3]. The mechanism by which low concentrations of Hg elicit these insulin-like effects is poorly understood, but it does not appear to involve interactions with the insulin receptor since exposure to Hg did not alter phosphorylation of the receptor or of insulin receptor substrate 1 [5]. Similar insulin-like effects not involving the insulin receptor have

* Corresponding author. Fax: +1-501-575-7139; E-mail: dbarnes@comp.uark.edu

0005-2736/99/$ – see front matter
PII: S0005-2736(99)00077-2

also been reported for other metals including zinc [4], cadmium [5], selenium [6], and vanadium [7].

In the present study, we used the *Xenopus laevis* oocyte model to examine the effects of Hg on hexose transport. *Xenopus* oocytes respond to a wide range of polypeptide and steroid hormones and have been used extensively to study the hormonal control of cell function. This very large cell facilitates microinjection, and analysis of metabolism and transport at the single cell level. We previously used the *Xenopus* oocyte model to examine the mechanisms by which insulin and insulin mimics, such as vanadate, control hexose transport and protein synthesis [8]. We show here that $HgCl_2$ had complex effects on hexose uptake, altering both the carrier-mediated and diffusive components. At low concentrations, Hg, like insulin, increased mediated transport through a change in the apparent V_{max}. Both insulin and Hg also increased cell volume. Swelling oocytes by exposure to hypotonic medium also increased the mediated hexose uptake, primarily through an increase in V_{max}. Together these data show that $HgCl_2$ affects several aspects of membrane function; they suggest that cell swelling could trigger the increase in hexose transport seen with insulin and Hg.

2. Materials and methods

2.1. Reagents

Bovine pancreatic insulin (25.7 U/mg) and mercuric chloride were purchased from Sigma (St. Louis, MO). [^{3}H]2-Deoxyglucose (>30 Ci/mmol), [^{14}C]polyethylene glycol (1.1 mCi/g, MW 4000), and [^{3}H]mannitol (>30 Ci/mmol) were obtained from DuPont-New England Nuclear (Boston, MA). All other chemicals were reagent grade or higher and obtained from commercial sources.

2.2. Oocytes

Dumont stage V oocytes (900–1100 μm diameter) with intact follicles were isolated manually from unstimulated *Xenopus laevis* (Xenopus One, Ann Arbor, MI) using watchmaker's forceps and fine scissors as described previously [9]. An amphibian oocyte Ringer (OR2, in mM: 82.5 NaCl, 2.5 KCl, 1.0 Na_2HPO_4, 1.0 $CaCl_2$, 1.0 $MgCl_2$, 1.0 Na pyruvate, and 5.0 HEPES, at pH 7.6) was the extracellular buffer solution; during incubations, 0.5 ml of buffer was used per cell. The data shown are from a single animal and are representative of experiments using cells from 3–5 animals. Incubations were carried out at 22°C.

Compounds were microinjected into oocytes in a 16 nl volume of intracellular buffer (nRP, containing in mM: 1.0 KH_2PO_4, 125 KCl, 10 NaCl, and 2.0 $NaHCO_3$, at pH 7.5) as previously described [10]. Cells were injected in the vegetal hemisphere using a glass micropipet (tip diameter <10 μm) connected to a hydraulic nanoliter pump (WPI Instruments, Sarasota, FL).

2.3. 2-Deoxyglucose (DG) uptake

Uptake of the model substrate DG was determined as previously described [8]. Briefly, oocytes were incubated in OR2 containing 200 μM [^{3}H]DG (4 μCi/ml) for 0.5 or 1.0 h depending on experiment. Cells were removed from labeling media, rapidly washed three times with deoxyglucose-free OR2 and solubilized with 1 N NaOH for liquid scintillation counting. Uptake (pmol/cell) was calculated from dpm/cell and medium-specific activity. We have previously shown that in the *Xenopus* oocyte, (1) over 90% of DG taken up by the oocytes was found in the oocyte rather than the follicle, (2) the rate of deoxyglucose uptake was constant for at least 4 h, and (3) in control cells, 80–90% of deoxyglucose uptake was mediated by an insulin-sensitive carrier, with the remainder being non-mediated.

2.4. Cell water content

Oocytes were incubated in modified OR2 (addition of $HgCl_2$ or diluted with H_2O). After 2 h, 5 μCi/ml [^{3}H]mannitol was added as a marker for trapped medium. The cells were rapidly weighed, air dried overnight in the presence of silica and Drierite ($CaSO_4$), reweighed, and amount of radiolabel determined by liquid scintillation counting as described above. Cellular water content was calculated using the following equation:

water content (μg H_2O/μg dry wt.)

$$= \frac{[\text{cell wet wt.} - \text{dpm}/(\text{dpm}/\mu\text{l medium})]}{(\text{cell dry wt.})}$$

3. Results

3.1. The $HgCl_2$ dose response

Fig. 1 shows the effects of increasing $HgCl_2$ concentration on 200 μM DG uptake. The dose-response relationship was complex, with three distinct types of effect. Low concentrations of $HgCl_2$ (0.1–10 μM) significantly increased DG; intermediate concentrations (30–50 μM) markedly reduced DG uptake; and concentrations greater than 100 μM increased uptake over control values. For the low Hg concentration range, maximal stimulation was found with 10 μM $HgCl_2$ and the effect was equivalent to that seen with 70–140 nM insulin (3–5-fold increase [8] and unpublished data). With 10 μM $HgCl_2$, significant stimulation of DG uptake was observed after 15–30 min of exposure and stimulation increased during 2 h of exposure (Fig. 1, inset); with 100 μM $HgCl_2$, 1–2 h of exposure were required before significant increases in DG uptake were evident (not shown).

To examine the reversibility of $HgCl_2$ effects on DG uptake, cells were exposed to 10 μM $HgCl_2$ for 15 min and then placed in Hg-free medium. DG uptake was measured following 105 additional minutes and was found to be more than double the control value (18.6 ± 2.8 pmol/cell in Hg-exposed cells vs. 8.6 ± 0.7 pmol/cell in control cells). Thus, a transient exposure was sufficient to elicit an effect, albeit somewhat reduced from that seen with a full 2 h exposure (Fig. 1). Addition of sulfhydryl reagents, such as dithiothreitol (DTT) or glutathionine, to the Hg-free medium after the initial (15 min) $HgCl_2$ exposure failed to prevent the increase in DG uptake measured 105 min later. However, when oocytes were exposed to 10 μM $HgCl_2$ and 1.0 mM DTT during the initial 15 min, DG uptake did not increase (not shown).

Previous studies from this laboratory and others have shown that DG uptake in *Xenopus* oocytes occurs by two processes: (1) simple diffusion, and (2)

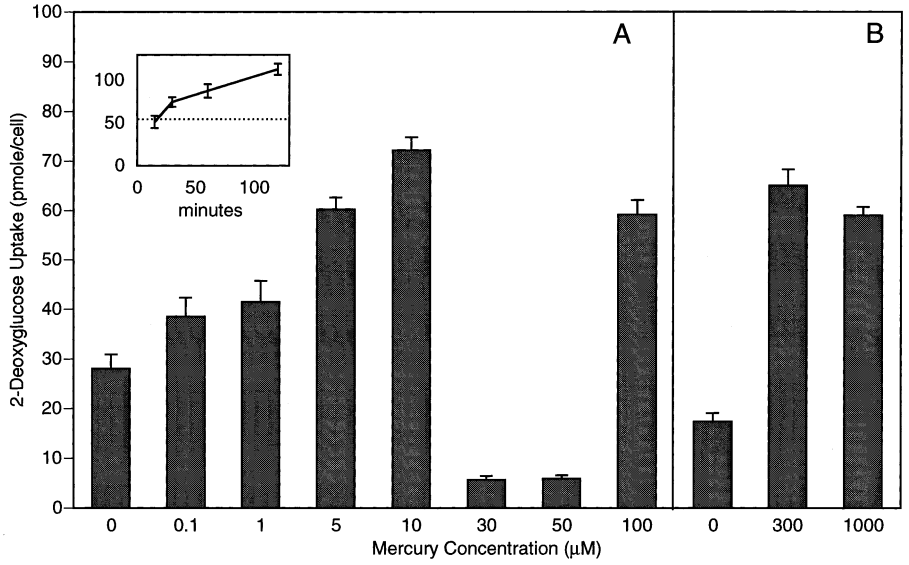


Fig. 1. Stimulation of 200 μM [^{3}H]DG uptake by mercuric chloride. Oocytes were incubated for 2 h in OR2 buffer with the mercury concentrations indicated. Hexose uptake was measured during the last 30 min of the incubation period. Panel A represents a low mercury concentration range and panel B a high concentration range. Inset in panel A shows the time course of 10 μM $HgCl_2$ action; the line represents hexose mean uptake for control cells. Data are given as the mean value for 9–10 cells with variability shown as S.E.M. bars. Statistical comparisons: hexose uptake in cells treated with 1.0, 5.0, 10, 100, 300 and 1000 μM mercury was significantly higher than corresponding controls ($P < 0.01$). Hexose uptake in cells treated with 50 μM $HgCl_2$ was significantly lower than control uptake ($P < 0.01$).

Table 1
Effect of $HgCl_2$ on the non-mediated and mediated components of [^{3}H]DG uptake

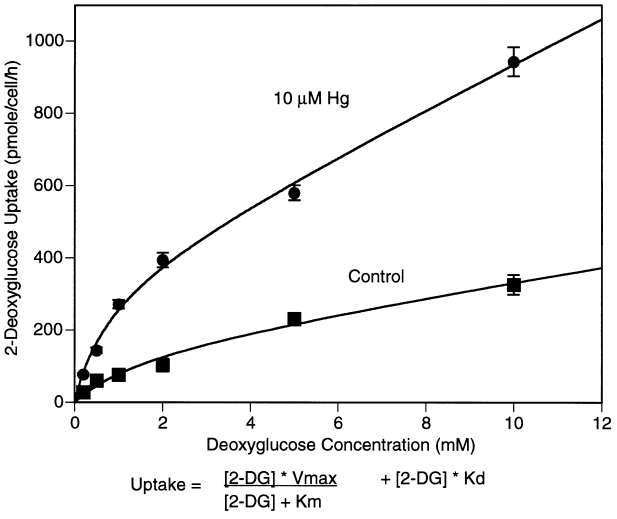
μM Hg	Total uptake	Non-mediated uptake	Mediated uptake
0	45.3 ± 7.7	8.8 ± 0.7	36.5 ± 7.7
10	122.6 ± 4.2*	15.9 ± 0.9*	106.7 ± 4.3*
50	19.7 ± 1.3*	6.1 ± 0.3*	13.6 ± 1.3*
0	35.2 ± 2.4	13.9 ± 1.0	21.3 ± 2.6
300	62.9 ± 4.0*	61.4 ± 3.3*	1.5 ± 5.2*
1000	64.4 ± 3.6*	59.4 ± 2.6*	5.0 ± 4.4*

Oocytes were incubated for 2 h in OR2 buffer containing the indicated Hg concentrations. Total hexose uptake was measured during the last 30 min of the incubation period with the addition of 200 μM [^{3}H]2-DG.
Non-mediated uptake was measured during the last 30 min of the incubation period with 200 μM [^{3}H]2-DG in the presence of 20 mM 2-DG. Data are given as the mean ± S.E.M. for 9–10 cells.
*Significantly different from corresponding untreated control value, $P < 0.01$.

carrier-mediated uptake, which is both specific and saturable [8,11]. To determine how these processes were altered by $HgCl_2$, we measured the effects of several $HgCl_2$ concentrations on 200 μM [^{3}H]DG uptake in the absence and presence of 20 mM unlabeled DG. This concentration of unlabeled DG is approximately 10–20 times the Michaelis constant (K_m) for DG uptake in oocytes [8,11] and thus should competitively block at least 90% of carrier-mediated [^{3}H]DG uptake. Using this procedure, we separated total [^{3}H]DG uptake into two components: a component that was insensitive to 20 mM DG, primarily representing the non-mediated component, and a second component that was blocked by 20 mM DG, approximating the carrier-mediated component of DG uptake. Table 1 shows that in control oocytes 80–85% of total DG uptake was carrier-mediated. In oocytes exposed to 10 μM $HgCl_2$, total DG uptake tripled. This increase was primarily due to a tripling of the mediated component of uptake, although the non-mediated component did increase significantly. With 50 μM $HgCl_2$, total uptake fell by more than 50% and this was primarily the result of a 65% decrease in the mediated component. With 300 and 1000 μM $HgCl_2$, total DG uptake nearly doubled and non-mediated uptake increased four-fold; mediated uptake fell by over 80%. These results indicate that: (1) at low concentrations, $HgCl_2$ stimulated DG uptake primarily by increasing the mediated component of uptake, (2) at intermediate concentrations, $HgCl_2$ reduced DG uptake primarily by decreasing mediated uptake, and (3) at high concentrations, $HgCl_2$ stimulated DG uptake by increasing the non-mediated component and nearly abolishing the mediated component.

3.2. The mediated component of DG uptake

To further characterize the effects of low concentrations of Hg on hexose transport, initial rates of



	Vmax (pmole/cell/h)	Km (mM)	Kd (pmole/cell/h)
Control	222 ± 12	1.3 ± 0.1	29 ± 10
10 μM Hg	1430 ± 70	2.6 ± 0.2	40 ± 4

Fig. 2. Kinetics of [^{3}H]DG uptake in the absence (control) and presence of 10 μM $HgCl_2$. Cells were incubated for 2 h in OR2 buffer with the indicated concentrations of DG. Incubations were carried out in the presence and absence of 10 μM $HgCl_2$. Hexose uptake was measured during the last h of the incubation period. Each data point represents the mean value for 9–10 oocytes with variability as shown as S.E.M. bars. Hexose uptake by the oocyte was represented by an equation that included uptake by simple diffusion plus one carrier-mediated term. Apparent maximum velocity (V_{max}), the apparent Michaelis constant (K_m) and the diffusion coefficient (K_{dif}) were calculated using non-linear, least-squares analysis of the velocity versus substrate concentration data (MacCurveFit 1.05). The curves shown were generated from the equation and the calculated constants.

DG uptake were measured in control oocytes and in oocytes exposed to 10 μM $HgCl_2$. Fig. 2 shows rates of DG uptake as a function of DG concentration as well as the calculated kinetic constants for the mediated and non-mediated components of uptake. As before [8], the data were fit to an equation that included terms for a single mediated component and passive diffusion. The kinetic constants reported here for control oocytes agree with those from previous studies [8,11]. The data show that $HgCl_2$ increased apparent maximum velocity (V_{max}) 6-fold, increased apparent K_m 2-fold, but had no significant effect (Welch *t*-test) on the diffusion coefficient (K_{dif}).

The large increase in apparent V_{max} for DG uptake caused by 10 μM Hg could have been due to an increase in carrier turnover or an increase in the number of plasma membrane carriers. To determine whether $HgCl_2$ effects were due to recruitment of hexose transporters, oocytes were treated with 10 μM phenylarsine oxide (PAO) in the absence and presence of 10 μM $HgCl_2$ or 140 nM insulin and measured DG uptake. Phenylarsine oxide has previously been used as an inhibitor of insulin-activated recruitment of hexose transporters to the plasma membrane [8,12]. Table 2 shows that PAO had a small stimulatory effect on DG uptake in control cells. We previously found a similar small, but significant, effect of PAO alone on DG uptake [8]. In the presence of PAO, insulin-mediated DG uptake was reduced by 95% and the $HgCl_2$-induced DG uptake was abolished. Together, these data indicate that low concentrations of $HgCl_2$, like insulin, increased hexose uptake by recruiting glucose transporters to the plasma membrane.

Table 2
Effect of phenylarsine oxide (PAO) on basal, Hg- or insulin-stimulated DG uptake

	Control	5 μM PAO
Control	13.8 ± 2.2	27.3 ± 2.3
10 μM Hg	91.5 ± 3.5*	24.7 ± 3.3*
140 nM insulin	144.3 ± 12.3*	39.2 ± 3.5*

Oocytes were incubated for 2 h in OR2 buffer containing the indicated additions. Hexose uptake was measure during the last 30 min of the incubation period. Data are given as the means ± S.E.M. for 9–10 cells.
*Significantly greater than corresponding control value, $P < 0.01$.

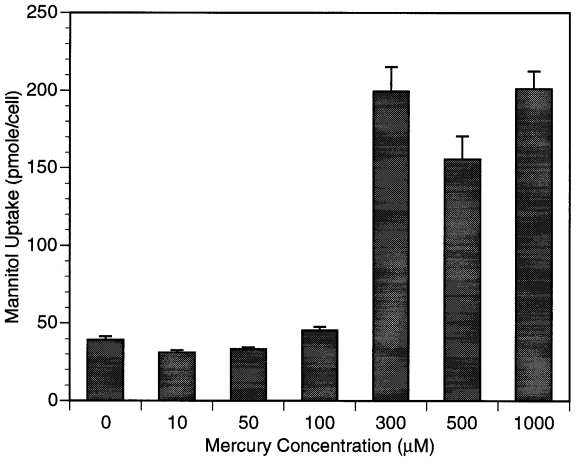


Fig. 3. Stimulation of 1.0 mM [^{14}C]mannitol uptake by mercuric chloride. Oocytes were incubated for 2 h in OR2 buffer with the mercury concentrations indicated. Mannitol uptake (1.0 μCi/ml 1.0 mM mannitol) was measured during the last 30 min of the incubation period. Data are given as the mean value for 9–10 cells with variability shown as S.E.M. bars. Statistical comparisons: mannitol uptake in cells treated with 300–1000 μM mercury was significantly higher than controls ($P < 0.01$).

Because of the time course of $HgCl_2$ action (Fig. 1, inset) and the 2 h incubation period used in most of the present experiments, changes in membrane carrier content could be due to insertion of newly synthesized carriers as well as insertion of already synthesized carriers. Indeed, we have found that in *Xenopus* oocytes, 10 μM $HgCl_2$ increases the rate of protein synthesis (methionine incorporation) 3–5-fold and that this increase in synthesis occurs over the same time frame as the increase in Hg-induced hexose transport (Barnes et al., unpublished data). To determine whether the increase in transport could be ascribed to insertion of newly synthesized carriers, we measured effects of cycloheximide pretreatment on DG uptake in control cells and cells exposed to 10 μM $HgCl_2$. Preliminary experiments showed that exposing oocytes to 20 μM cycloheximide blocked 75% of basal methionine incorporation into protein in control oocytes and 90% of methionine incorporation in mercury-treated oocytes. Deoxyglucose uptake in control and $HgCl_2$-treated cells averaged 13.5 ± 2.2 and 129.6 ± 8.2 pmol/cell respectively. With cycloheximide pretreatment, DG uptakes were 16.6 ± 1.3 and 109.8 ± 3.7 pmol/cell for control and Hg-treated cells, respectively. Thus, inhibition of protein synthesis had little effect on rates of hexose uptake.

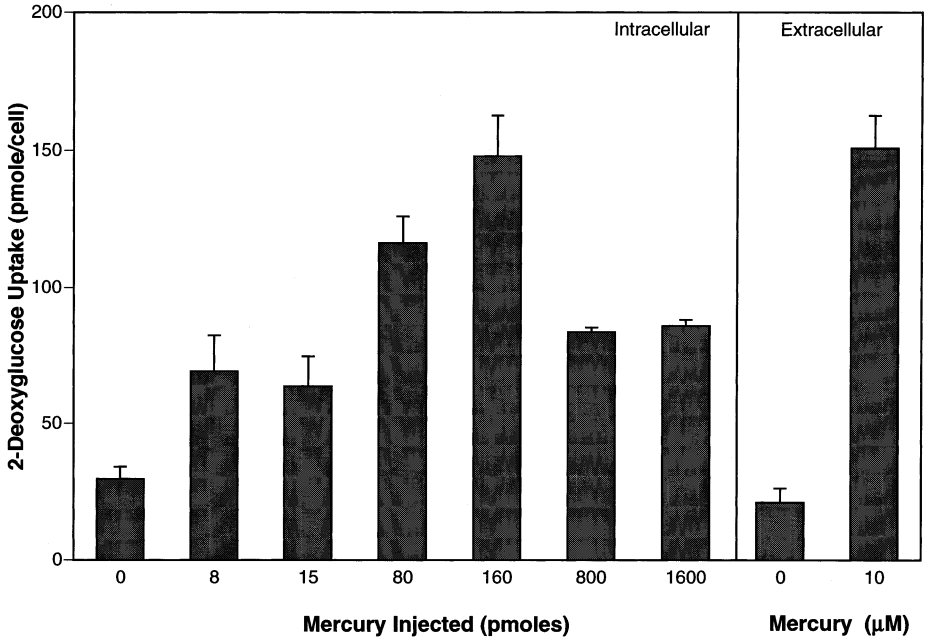


Fig. 4. Effect of microinjected $HgCl_2$ on 200 μM [^{3}H]DG uptake. Cells in OR2 were microinjected with 16 nl of intracellular buffer containing the indicated dose of $HgCl_2$. After 1 h, cells were transferred to OR2 buffer with labeled hexose. One hour later, they were processed to measure hexose uptake. Data are given as the mean value for 9–10 cells with variability shown as S.E.M. bars. All Hg concentrations significantly stimulated DG uptake ($P < 0.05$).

3.3. The non-mediated component of DG uptake

Further evidence for high concentrations of $HgCl_2$ increasing oocyte membrane passive permeability came from two types of experiments. First, we measured uptake of [^{14}C]mannitol, a solute with no known specific transporter and that shows no saturation of transport over a several order of magnitude concentration range (data not shown). Fig. 3 shows that 10 μM and 50 μM $HgCl_2$ had little or no effect on mannitol uptake, however, Hg concentrations higher than 100 μM increased mannitol uptake 5-fold. Second, if increased passive plasma membrane permeability was the underlying cause of the increased uptake at high $HgCl_2$ concentrations, then solute efflux as well as solute uptake should also be increased. To test this, [^{14}C]polyethylene glycol (PEG, MW 4000 Da) was microinjected into oocytes and the rate of tracer efflux into the medium measured. After 1 h, control oocytes lost $1.9 \pm 0.2\%$ of injected label and oocytes exposed to 300 μM $HgCl_2$ lost $15.9 \pm 2.0\%$, an apparent 8-fold increase in permeability to PEG. The mannitol uptake and PEG efflux data are consistent with high concentrations of $HgCl_2$ increasing the passive permeability of the oocyte plasma membrane.

3.4. Sites of $HgCl_2$ action

$HgCl_2$ readily permeates cell membranes and thus could potentially interact with both extracellular and intracellular sites [13]. Fig. 1 and Table 1 indicate that $HgCl_2$ has at least three distinct effects on transport: stimulation of mediated uptake at low concentrations, inhibition of mediated uptake at intermediate to high concentrations, and increased non-mediated uptake at high concentrations. To determine whether intracellular sites could be responsible for these effects, we microinjected $HgCl_2$ into oocytes and measured DG uptake. Fig. 4 shows that microinjected $HgCl_2$ did indeed increase DG uptake, with doses as low as 8 pmol (8 μM assuming 1.0 μl of cell water and all Hg remains in solution) significantly stimulating DG uptake. Maximal stimulation was seen with 160 pmol (160 μM) and the magnitude of this stimulation was comparable to that seen with 10 μM external $HgCl_2$. With higher injected doses of $HgCl_2$, stimulation of DG uptake fell off. How-

Table 3
Effect of microinjected $HgCl_2$ on total, mediated and non-mediated [^{3}H]DG uptake

pmol Hg	Total uptake	Non-mediated uptake	Mediated uptake
0	16.2 ± 3.9	6.5 ± 1.0	9.7 ± 4.0
16	32.9 ± 4.9*	10.2 ± 1.2*	22.7 ± 5.0
160	69.0 ± 1.3*	13.2 ± 1.6*	55.8 ± 2.1*
800	62.0 ± 1.0*	26.9 ± 2.5*	35.1 ± 2.7*
1600	67.4 ± 2.9	28.9 ± 3.9*	38.5 ± 4.9*

Oocytes were microinjected with the Hg doses indicated and incubated with 0.2 mM DG (total uptake) or 20 mM DG (non-mediated uptake). Hexose uptake was measure during the last 30 min of a 2 h incubation period. Data are given as the means ± S.E.M. for 9–10 cells.
*Significantly greater than corresponding control value, $P < 0.01$.

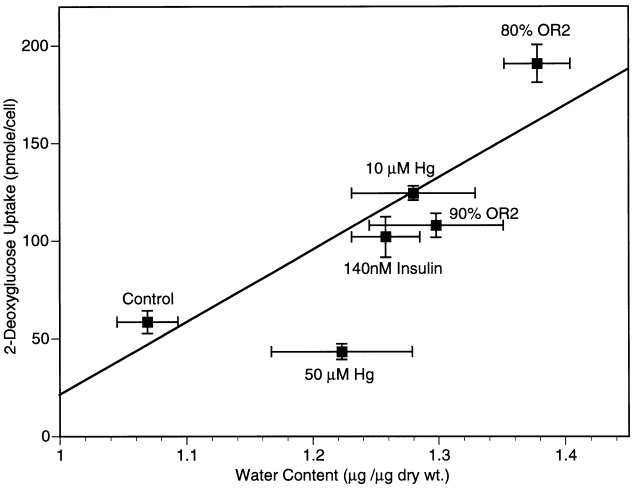


Fig. 5. Relationship between deoxyglucose uptake and cell water content. Deoxyglucose uptake is plotted as a function of cell swelling or water content. The the regression line was determined using all but the 50 μM Hg point ($r^2 = 0.81$).

ever, even doses as high as 1600 pmol still increased DG uptake significantly (Fig. 4). At all doses tested, $HgCl_2$ increased transport through the mediated component, i.e. the component that could be blocked by 20 mM cold DG (Table 3). However, the highest doses of injected $HgCl_2$ also significantly increased uptake through the non-mediated component.

3.5. $HgCl_2$ action and cell swelling

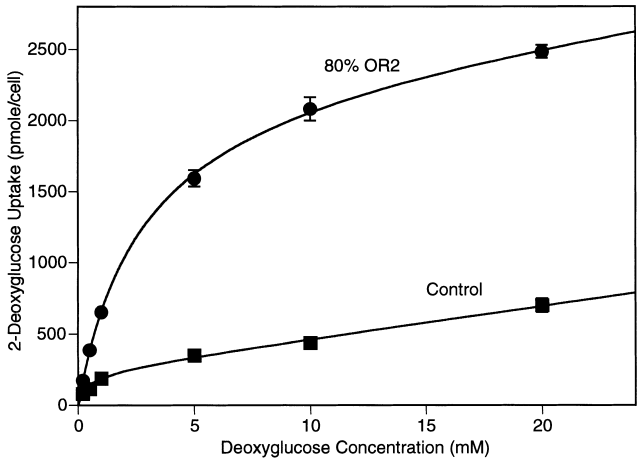
Osmotic swelling of cells has been shown to alter a variety of metabolic endpoints including, glycogen synthesis, gluconeogensis, amino acid transport and proteolysis, and changes in cell volume appear to be early events in the actions of certain hormones, including, insulin [14]. In this regard, $HgCl_2$ is a potent inhibitor of plasma membrane ion-transporting ATPases and a disrupter of ion channel function, both of which can lead to disturbed cell volume regulation, usually seen as cell swelling [1,13,15]. To determine whether changes in cell volume were involved in the action of $HgCl_2$ on DG transport in oocytes, we measured oocyte water content and DG uptake in cells exposed to medium with reduced osmolality, $HgCl_2$ or insulin.

The response of many small, somatic cells to osmotic challenge is biphasic. Initially, they behave as ideal osmometers as water rapidly flows down an osmotic gradient. Within minutes, volume regulatory processes are activated and cells begin to recover initial volume [16]. In contrast, *Xenopus* oocytes respond slowly to osmotic challenges. For example,

Table 4
Effects of osmotically induced cell swelling, $HgCl_2$ and insulin on oocyte water content and DG uptake

	μg H_2O/μg dry wt.	% Control	DG uptake (pmol/cell)	% Control
Control	1.069 ± 0.024		58.6 ± 5.8	
90% OR2	1.298 ± 0.053**	121	107.9 ± 6.2**	184
80% OR2	1.378 ± 0.026**	129	190.8 ± 9.7**	325
140 nM insulin	1.258 ± 0.027**	118	102.0 ± 10.4**	174
10 μM $HgCl_2$	1.280 ± 0.049**	120	124.5 ± 3.7	212
50 μM $HgCl_2$	1.223 ± 0.056*	114	43.4 ± 4.0	74

Oocytes were incubated for 2 h in OR2 buffer containing the indicated modifications. Hexose uptake was measured during the last 30 min of the incubation period. Data are given as the means ± S.E.M. for 9–10 cells.
*Significantly greater than control value, $P < 0.05$.
**Significantly greater than corresponding control value, $P < 0.01$.



	Vmax (pmole/cell/h)	Km (mM)	Kd (pmole/cell/h)
Control	242 ± 32	5.2 ± 1.9	23 ± 2
80% OR2	2322 ± 122	2.7 ± 0.2	22 ± 5

Fig. 6. Kinetics of [^{3}H]DG uptake under control and hypotonic conditions. Cells were incubated for 2 h with the indicated concentrations of labeled hexose and either complete OR2 or 80% OR2. Hexose uptake was measured during the last hour of the incubation period. The equation models uptake by simple diffusion plus one carrier-mediated term. Apparent maximum velocity (V_{max}), the apparent Michaelis constant (K_m) and the diffusion coefficient (K_{dif}) were calculated using non-linear, least-squares analysis of the velocity versus substrate concentration data (MacCurveFit 1.05). The curves shown were generated from the equation and the calculated constants. Each point represents the mean value for 9–10 oocytes; variability is shown as S.E.M. bars.

when medium osmolality was reduced, oocytes swelled and did not appear to subsequently volume regulate. Table 4 shows that 2 h after transfer to hypotonic medium, oocyte water content was still elevated. Table 4 also shows that, over the osmolality range studied, DG uptake increased with oocyte water content. Other experiments demonstrated that the increase in DG uptake was detectable 15 min after transfer to hypotonic medium and was maximal within 30 min; when oocytes were removed from hypotonic medium (80%) after 15 min and placed in unaltered medium they recovered initial volumes and basal levels of DG uptake. Hypertonic medium (addition of 40 mM mannitol) reduced DG uptake slightly but had no effect on Hg-stimulated DG uptake (data not shown).

$HgCl_2$ (10 μM) and insulin also increased water content significantly (Table 4). These increases in water content occurred in isotonic medium. They were also accompanied by substantial increases in total DG uptake. However, when the Hg concentration was raised to 50 μM, cell water content increased, but DG uptake did not (Table 4). We used the data in Table 4 to plot DG uptake as a function of cell water content (Fig. 5). Except for the 50 μM Hg point, the data show a remarkable correlation between the two variables. Increasing Hg concentration caused no further swelling but did increase hexose uptake by non-mediated pathways.

To further characterize the swelling-induced increase in DG transport, initial rates of DG uptake were measured as a function of substrate concentration in oocytes maintained in OR2 medium or in hypotonic medium (80% OR2). Hypotonic medium increased DG uptake at all DG concentrations studied. Swelling increased apparent V_{max} 9-fold and apparent K_m 3-fold but had no effect on K_{dif} (Fig. 6). Note that, as with 10 μM Hg and insulin, the primary effect of cell swelling was on the V_{max} for the mediated component of DG uptake.

4. Discussion

The results of the present study show that $HgCl_2$ can alter *Xenopus* oocyte membrane transport function in several ways, and that the nature of the effect that one sees depends on the Hg concentration to which the cells are exposed. This can best be seen in the Hg dose response data shown in Fig. 1 and Table 1. The data for total DG uptake gave the appearance of a triphasic curve. That is, total uptake increased with low Hg concentrations, fell with intermediate concentrations and again increased with higher concentration. When total uptake was separated into mediated and non-mediated components using high concentrations of unlabeled DG, two distinct dose-response patterns emerged. Non-mediated uptake was not substantially affected by low Hg concentrations, fell with intermediate concentrations and increased markedly with high concentrations. Experiments in which mannitol uptake and PEG efflux were

measured confirmed the increase in plasma membrane passive permeability caused by high $HgCl_2$ concentrations. In contrast, mediated uptake of DG increased with low $HgCl_2$ concentrations, and then fell with intermediate and high concentrations. With $HgCl_2$ concentrations greater than 100 μM, mediated uptake was less than 20% of control values. Together, these dose response data indicate that $HgCl_2$ has four distinct effects on DG uptake in these oocytes: a stimulation then an inhibition of mediated DG uptake; a decrease then an increase in non-mediated uptake.

Of the four effects, clearly the most interesting is the increase in mediated DG uptake seen with low Hg concentrations. Kinetic analyses demonstrated that 10 μM Hg increased the apparent V_{max} 6-fold and the apparent K_m 2-fold. Similar large increases in the apparent V_{max} for DG uptake have been found in oocytes exposed to insulin and to insulin mimics, e.g. pervanadate, and these have been shown to involve recruitment and insertion of transporters into the plasma membrane [8]. The present experiments show that PAO blocked the stimulation of DG uptake by 10 μM Hg, a result that indicates that the increase in apparent V_{max} caused by Hg was due to transporter insertion. The absence of effect of cycloheximide on basal or Hg-stimulated transport indicates that newly synthesized transporters were most likely not involved to any appreciable extent. The inability of the small hydrophilic sulfhydryl reagents to reverse the early effects of mercury suggests tight binding and/or intracellular sites of action.

$HgCl_2$ is sufficiently lipophilic so that one cannot use its time course of action to predict whether Hg interactions with internal or external sites are responsible for a given effect. Because of their large size, *Xenopus* oocytes allow one to quantitatively microinject compounds directly into the cytoplasm with minimal damage to the cells. To determine whether internal sites of action were involved in the Hg effects on DG transport, we microinjected $HgCl_2$ into oocytes and measured uptake of DG. Two types of responses were seen. With all doses of injected Hg, total DG uptake increased. At low doses, the increase was entirely due to increased mediated uptake; at higher doses, both mediated and non-mediated uptake had increased. Our calculations show that these effects cannot be attributed to leakage of injected Hg into the medium. For example, with 160 pmol injected, if all the Hg had leaked out of the cell, the medium concentration would only have been 0.3 μM. The dose response data in Fig. 1 indicate that this Hg concentration would be expected to increase DG uptake only slightly, not by nearly 4-fold as was seen in the microinjection experiment. Similarly, with 1600 pmol of injected Hg, total leakage would have resulted in a medium Hg concentration of 3 μM, which would have been too low to increase the non-mediated component of DG uptake. These data argue that, at a minimum, Hg can act at internal sites to affect both the mediated and non-mediated components of DG uptake.

It is generally accepted that cell volume plays some role in the regulation of cellular metabolism. It is also clear that, through its actions on metabolite and ion transport, Hg alters cell volume. Previous reports have indicated that osmotically induced cell swelling mimics many of insulin's metabolic actions [17,18] and that conversely insulin causes cell swelling [14]. Incubation in hypotonic media, like insulin stimulation, increases glycogen synthesis, increases glycolysis and decreases gluconeogenesis and proteolysis. The present results show that hypo-osmotic conditions, low concentrations of Hg, and insulin all increased cellular water content and hexose uptake, the latter primarily through an increase in the apparent V_{max}. For Hg and insulin, insertion of hexose transporters into the plasma membrane appears to underlie the kinetic effect [19], and present study. We do not yet know whether this is also the case for cell swelling. Since water transport has been reported through hexose transporters it is possible that cell swelling observed for insulin and Hg is mediated by increased hexose transporter number [20]. Evidence from osmotically swelled cells suggest that swelling preceded the increase in hexose uptake and that insulin- and Hg-induced swelling could precede transporter recruitment. These finding suggest that cell swelling, low concentrations of Hg and insulin might stimulate transport through a common mechanism. The rapid increase in cell volume that accompanies Hg and insulin stimulation of DG uptake could be one link in the chain that signals this biochemical effect.

Insulin affects a multitude of cellular events including membrane transport, enzyme activity, gene ex-

pression, protein synthesis and DNA synthesis [19]. The inability to properly maintain glucose homeostasis, whether due to an interruption in the production of insulin by the pancreas, or to altered cellular responsiveness, is the most obvious symptom of diabetes. At low concentrations, group IIb metals, mercury, cadmium and zinc, stimulate several insulin-regulated metabolic processes and thus may have the capacity to disrupt normal insulin metabolism. These metals exhibit insulin-like and insulin-potentiating effects both in vitro and in vivo, including stimulation of RNA synthesis, stimulation of protein synthesis, and alterations of hexose transport and metabolism [5,21–23]. The insulin-like actions of these metals may not be limited by normal control mechanisms, since evidence suggests they act downstream from the insulin receptor [5]. As with the steroid-like endocrine disrupters, mercury and cadmium persist within the body, effectively increasing the duration of exposure and the potential for cumulative effects. Therefore, mercury and other group IIb metals may represent environmental pollutants capable of causing uncontrolled insulin-like effects that could result in a disruption of homeostasis and may complicate the effects of diabetes. As such, they represent another class of endocrine disrupters, with actions very different from those with steroid hormone-like effects.

Acknowledgements

This study was supported, in part, by Grant R825218-01-0 to D.M.B. from the United States Environmental Protection Agency.

References

[1] A.E. Shamoo, in: C.U. Eccles, Z. Annau (Eds.), The Toxicity of Methyl Mercury, The John Hopkins University Press, Baltimore, MD, 1987, pp. 13–23.
[2] P.E. Brubaker, S.P. Herman, G.W. Lucier, L.T. Alexander, M.D. Long, Exp. Mol. Pathol. 18 (1973) 263–280.
[3] G.J. Sauve, D.M. Nicholls, Int. J. Biochem. 13 (1981) 981–990.
[4] J.M. May, S. Contoreggi, J. Biol. Chem. 257 (1982) 4362–4368.
[5] O. Ezaki, J. Biol. Chem. 264 (1989) 16118–16122.
[6] O. Ezaki, J. Biol. Chem. 265 (1990) 1124–1128.
[7] Y. Shechter, J. Meyerovitch, Z. Farfel, J. Sack, R. Bruck, S. Bar-Meir, S. Amir, H. Degani, S.J.D. Karlish, in: N.D. Chasteen (Ed.), Vanadium in Biological Systems, Kluwer Academic, Dordrecht, 1990, pp. 129–142.
[8] D.M. Barnes, D.B. Sykes, Y. Shechter, D.S. Miller, J. Cell Physiol. 162 (1995) 154–161.
[9] D.S. Miller, Y.-T. Lau, S.B. Horowitz, Proc. Natl. Acad. Sci. USA 81 (1984) 1426–1430.
[10] D.S. Miller, Science 240 (1988) 506–509.
[11] M. Janicot, M.D. Lane, Proc. Natl. Acad. Sci. USA 86 (1989) 2642–2646.
[12] S.C. Frost, M.D. Lane, J. Biol. Chem. 260 (1985) 2646–2652.
[13] N. Ballatori, C. Shi, J.L. Boyer, Toxicol. Appl. Pharmacol. 95 (1988) 279–291.
[14] D. Haussinger, F. Lang, W. Gerok, Am. J. Physiol. 267 (1994) E343–E355.
[15] B.M. Anner, M. Moosmayer, Am. J. Physiol. 262 (1992) F843–F848.
[16] D. Haussinger, F. Lang, Biochim. Biophys. Acta 1071 (1991) 331–350.
[17] G. Wu, N.E. Flynn, Biochim. Biophys. Acta 1243 (1995) 343–350.
[18] M. Al-Habori, M. Peak, T.H. Thomas, L. Agius, Biochem. J. 282 (1992) 789–796.
[19] B. Cheatham, C.R. Kahn, Endocr. Rev. 16 (1995) 117–142.
[20] D.D. Loo, T. Zeuthen, G. Chandy, E.M. Wright, Proc. Natl. Acad. Sci. USA 93 (1996) 13367–13370.
[21] S.A. Harrison, J.M. Buxton, B.M. Clancy, M.P. Czech, J. Biol. Chem. 266 (1991) 19438–19449.
[22] H. Inamoto, Y. Ino, N. Inamoto, T. Wada, H.K. Kihara, I. Watanabe, S. Asano, Lab. Invest. 34 (1976) 489–494.
[23] A. Shisheva, Y. Shechter, Endocrinology 133 (1993) 1562–1568.