Uncoupling of Attenuated *myo*-[^{3}H]Inositol Uptake and Dysfunction in Na^+-K^+-ATPase Pumping Activity in Hypergalactosemic Cultured Bovine Lens Epithelial Cells

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Attenuation of both the active transport of *myo*-inositol and Na^+-K^+-ATPase pumping activity has been implicated in the onset of sugar cataract and other diabetic complications in cell culture and animal models of the disease. Cultured bovine lens epithelial cells (BLECs) maintained in galactose-free Eagle's minimal essential medium (MEM) or 40 mM galactose with and without sorbinil for up to 5 days were examined to determine the temporal effects of hypergalactosemia on Na^+-K^+-ATPase and *myo*-inositol uptake. The Na^+-K^+-ATPase pumping activity after 5 days of continuous exposure to galactose did not change, as demonstrated by ^{86}Rb uptake. The uptake of *myo*-[^{3}H]inositol was lowered after 20 h of incubation in galactose and remained significantly below that of the control throughout the 5-day exposure period. The coadministration of sorbinil to the galactose medium normalized the *myo*-[^{3}H]inositol uptake. No significant difference in the rates of passive efflux of *myo*-[^{3}H]inositol or ^{86}Rb from preloaded galactose-treated and control cultures was observed. Culture-media reversal studies were also carried out to determine whether the galactose-induced dysfunction in *myo*-inositol uptake could be corrected. BLECs were incubated in galactose for 5 days, then changed to galactose-free physiological medium with and without sorbinil for a 1-day recovery period. *myo*-Inositol uptake was reduced to 34% of control after 6 days of continuous exposure to galactose. Within 24 h of media reversal, *myo*-inositol uptake returned to or exceeded control values in BLECs switched to either MEM or MEM with sorbinil. These observations support the contention that the aldose reductase reaction or formation of the product of the aldose reductase reaction disrupted lens cell *myo*-inositol uptake. Although the coadministration of sorbinil to the galactose medium protected against the attendant decrease in transport activity, the cessation of exposure of BLECs to galactose was sufficient stimulus to normalize *myo*-inositol uptake. Moreover, these observations suggested that the deficit in *myo*-inositol uptake elicited by exposure to galactose was reversible and occurred independently of changes in Na^+-K^+-ATPase pumping activity in cultured lens epithelium, indicating that the two parameters are not strictly associated and that the deficit in *myo*-inositol uptake occurs rapidly during hypergalactosemia. *Diabetes* 40:731–37, 1991

Aldose reductase has been implicated in several related metabolic abnormalities believed to contribute to the pathogenesis of diabetic complications in the lens and other tissues. The accumulation of intracellular polyol content and the loss of Na^+-K^+-ATPase activity are thought to be associated with the early onset of diabetic complications (1–6). The underlying mechanism(s) leading to *myo*-inositol depletion are, to date, obscure. Tomlinson et al. (7) reported that the aldose reductase inhibitor sorbinil prevented and reversed the derangement of neuronal sorbitol and *myo*-inositol concentrations in streptozocin-induced diabetic (STZ-D) rats. In a related study, the membrane-bound stimulated Na^+-K^+-ATPase activity of the sciatic nerve from STZ-D rats decreased in parallel with the decrease in intraneuronal *myo*-inositol concentration (8). This appeared to be a consequence of the change in neuronal *myo*-inositol concentration, which was also preventable by sorbinil administration. Nerve conduction impairment and a reduction in sciatic nerve Na^+-K^+-ATPase activity in STZ-D rats were selectively prevented by administration of a 1% *myo*-inositol–supplemented diet (9). On the other hand, Kawaba et al. (10) reported that addition of excess *myo*-inositol to 30 mM galactose medium failed to correct the loss of *myo*-inositol and Rb-concentrating ability or the depressed Na^+-K^+-

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ATPase enzymatic activity in rat lenses maintained in organ culture. Yeh et al. (11) demonstrated that, although sorbinil treatment reversed the decrease in Na^+-K^+-ATPase transport activity observed in the intact lens of STZ-D rats, the *myo*-inositol concentration remained low. They concluded that the restoration of lenticular Na^+-K^+-ATPase transport activity was not secondary to a normalization of *myo*-inositol content, suggesting that these two parameters were not strictly associated in the diabetic lens. Because of the apparent link between *myo*-inositol metabolism and Na^+-K^+-ATPase activity, these studies were carried out to determine the nature of the association between the attenuation of *myo*-inositol–concentrating capability and Na^+-K^+-ATPase transport activity as measured by *myo*-inositol and Rb uptake with cultured bovine lens epithelial cells (BLECs) exposed to high ambient galactose.

RESEARCH DESIGN AND METHODS

Bovine (*Bos taurus*) eyes obtained from a local slaughterhouse were brought on ice to the laboratory, where the lenses were removed aseptically. After making incisions on each side of the equator, the anterior capsule of each lens with its epithelium attached was peeled away from the cortex and placed in a 60-mm petri dish in 5 ml of a growth medium composed of Eagle's minimal essential medium (MEM) supplemented with 10% calf serum, nonessential amino acids, 5 mg/L ascorbic acid, and 20 mg/L gentamycin sulfate and maintained in a water-humidified atmosphere of 5% CO_2/95% air at 37°C as previously described (12). This growth medium contains ~10–15 μM *myo*-inositol. Cell outgrowth after 7–10 days from the capsule to the petri dish was dispersed in Ca^{2+}-Mg^{2+}–free MEM containing 0.125% trypsin/0.05% EDTA and transferred to a 75-cm^2 culture flask. The cells originating from two to three capsules were placed in each culture flask with 40 ml of growth medium. On reaching confluence, the cells were again dispersed and subcultured in a split ratio of 1:10 in 25-cm^2 culture flasks containing 5 ml of growth medium. All studies were performed with confluent monolayers in 25-cm^2 culture flasks (representing 2nd-passage cells).

Metabolic radiolabeling was determined as follows: the cultured cells were divided into groups and the medium replaced with galactose-free physiological medium (5.5 mM glucose, MEM) or physiological medium containing 40 mM galactose (Sigma, St. Louis, MO) in the presence and absence of 1×10^{-4} M sorbinil. The cultures were preincubated for either 20 h or 5 days before the addition of the isotopes to either the control or galactose medium. The accumulation of *myo*-inositol and Rb was achieved by incubating the cultured cells in the presence of 0.2 μCi/ml medium *myo*-[^{3}H]inositol (30 Ci/mmol; American Radiolabeled Chemicals, St. Louis, MO) or ^{86}Rb (4.65 mCi/mg; Du Pont-NEN, Boston, MA) for up to 10 h or 90 min, respectively. After isotope incubation, the medium was removed, and the culture flasks were rinsed three times with ice-cold Ca^{2+}-added phosphate-buffered saline (137 mM NaCl, 8 mM dibasic sodium phosphate, 0.7 mM calcium chloride, pH 7.2) and drained overnight at 4°C. Five milliliters of 2% sodium carbonate in 0.1 N sodium hydroxide was added to each flask and left overnight at room temperature to insure cell lysis. Replicate 0.5-ml aliquots were taken for liquid-scintillation counting (Packard Tri-Carb 4640, Laguna Hills, CA). Triplicate 0.5-ml aliquots were taken for protein determination by the method of Bradford et al. (13) with bovine serum albumin (Sigma) as standard. Accumulation of *myo*-inositol or Rb was expressed as counts per minute (cpm) per milligram of protein in individual culture flasks.

For the measurement of passive efflux, BLECs were preloaded for 4 h with the appropriate medium in the presence of 0.2 μCi/ml of *myo*-[^{3}H]inositol or ^{86}Rb. After the load-up period, the culture flasks were rinsed with buffer as described above, and 5 ml of fresh medium was added. The efflux experiments were performed over a 4-h period, and for the ^{86}Rb efflux, 0.1 mM ouabain was added to each medium to inhibit the reentry of ^{86}Rb by active transport. The efflux was expressed as the percentage of radioactivity initially present in the cultured lens epithelium.

For medium-reversal studies, the cultured cells were separated into groups as above, the medium being replaced with unmodified MEM or galactose with and without sorbinil. The cultures were maintained for 5 days, at which time they were divided into two categories: *1*) either the conditioned medium was replaced with appropriate fresh medium (MEM or galactose ± sorbinil) or *2*) several of the cell populations previously exposed to galactose were reversed to galactose-free MEM ± sorbinil. The cultures were maintained under these conditions for an additional 20 h. Thereafter, the accumulation of *myo*-[^{3}H]inositol was performed as described above and expressed as counts per minute per milligram of protein in each culture flask.

Galactitol and *myo*-inositol determination was performed as follows. BLECs maintained in replicate 75-cm^2 culture flasks as described above were trypsinized, and the combined cell pellets were homogenized in 0.5 ml 0.3 N zinc sulfate mixed with an equal volume of 0.3 N barium hydroxide and centrifuged at 15,000 × *g* for 30 min. The clear supernatant was transferred to a 5-ml glass vial and lyophilized to dryness. Before analysis, the sample was reconstituted in 0.4 ml H_2O, and an aliquot was injected onto an HPIC-AS6 anion-exchange column (Dionex, Sunnyvale, CA) for quantitation. The microanalysis of cellular galactitol and *myo*-inositol was performed with a Dionex BioLC chromatographic system with a pulsed amperometric detector. The sample on the column was eluted with 45 mM NaOH at 0.5 ml/min. This system allows galactitol and *myo*-inositol to be detected with a sensitivity in the picomole range and was recently used to determine the *myo*-inositol content in cultured human lens epithelial cells (14).

Statistical analyses were performed with the statistical programs from Tallarido and Murray (15) adapted for the IBM PC-XT. Appropriate statistical analyses were applied to each group of data as indicated.

RESULTS

BLECs maintained in 40 mM galactose for 6 days prompted the synthesis and intracellular accumulation of galactitol (Table 1). The concomitant administration of sorbinil to the galactose medium during the 6-day exposure prevented the accumulation of galactitol, the galactitol concentration being undetectable with the Dionex BioLC chromatographic system. Determination of the *myo*-inositol content in cultured BLECs exposed to galactose-free physiological medium, 40

TABLE 1
Galactitol and *myo*-inositol content of cultured bovine lens epithelial cells (BLECs)

Treatment	Galactitol (mM)	*myo*-Inositol (mM)
MEM	0*	28
Galactose	1.54	30
Galactose + sorbinil	0*	25

Confluent monolayers of BLECs were incubated for 6 days in physiological Eagle's minimal essential medium (MEM), 40 mM galactose, and 40 mM galactose + sorbinil. The data represent duplicate determinations from individual flasks.
*<10 pmol, which is the limit of the Dionex BioLC chromatographic system with a pulsed amperometric detector.

mM galactose, or galactose with sorbinil for 6 days of continuous incubation displayed a concentration range of 25–30 mM, which did not change significantly under any of the treatment conditions (NS with the least significant difference test; Table 1).

We previously reported that incubation of BLECs in high ambient galactose medium (40 mM) for 6 days resulted in a significant lowering in the ability to concentrate *myo*-[^{3}H]inositol compared with cells maintained in galactose-free physiological medium. The simultaneous administration of sorbinil to the galactose medium protected against the observed galactose-induced decrease in *myo*-inositol uptake (16). In this study, the ability to accumulate *myo*-[^{3}H]inositol was investigated in BLECs maintained in high ambient galactose for acute (20-h) and chronic (5-day) periods of exposure so that the temporal sequence between the attenuation of the active transport of *myo*-inositol and the loss of Na^+-K^+-ATPase pumping activity, as demonstrated by Rb uptake, could be established. At the end of either incubation period, the medium was replaced with 40 mM of fresh galactose containing a tracer concentration of *myo*-[^{3}H]inositol (0.2 μCi/ml of medium), and incubation of the lens cell cultures continued for up to 10 h. Replicate flasks were collected at the end of the 1st and 2nd h and every 2 h thereafter, and the cell cytosolic counts per minute were determined. Figure 1 shows that exposure of the cultured cells to 40 mM galactose for as little as 20 h resulted in an attenuation of the ability to accumulate radiolabeled *myo*-inositol (as demonstrated by the cytosolic cpm/mg protein). The capability of the galactose-exposed cells to accumulate *myo*-[^{3}H]inositol remained significantly below that of the controls throughout the 5-day exposure period ($P < 0.01$ for difference from MEM with a linear regression comparison and a test for parallelism). The addition of ouabain completely arrested the ability to concentrate *myo*-inositol ($P < 0.01$ for differences from MEM).

The measurement of Na^+-K^+-ATPase pumping activity, as demonstrated by Rb uptake after 5 days of continuous exposure to galactose, did not appear to be adversely affected, the resulting curve being virtually indistinguishable from the one generated with the cells maintained in physiological medium (Fig. 2). The addition of sorbinil to either the galactose medium or the galactose-free physiological medium did not alter the rate of Rb uptake. The addition of ouabain substantially reduced Rb uptake in both hypergalactosemic and control cultures ($P < 0.01$ for differences from MEM- and galactose-treated cells by linear regression comparison and a test for parallelism).

To determine if the decrease in *myo*-inositol uptake was due to an enhanced efflux from the cells to the media, BLECs were preloaded with *myo*-[^{3}H]inositol, and the rate of efflux was determined. The rate of efflux of *myo*-inositol (Fig. 3) was virtually identical for cultured lens cells incubated in galactose with and without sorbinil and galactose-free physiological medium (NS by the least significant difference test). Likewise, the rate of efflux of Rb was similar under all treatment conditions (Fig. 4).

As described above, 5-day galactose-maintained BLECs displayed a marked decrease in ability to accumulate *myo*-[^{3}H]inositol relative to control cells (Fig. 5). The concomitant administration of sorbinil to the galactose medium completely prevented the attenuation of *myo*-inositol uptake. To determine if the galactose-exposed cells were permanently damaged, several cultures were switched to galactose-free MEM after 5 days of sustained galactose exposure in the presence and absence of sorbinil. The combined sorbinil and galactose-free medium restored the lens cell cultures' capacity to accumulate *myo*-inositol such that the rate of uptake was equal to or exceeded the rate of uptake observed with the cells continuously maintained in physiological me-

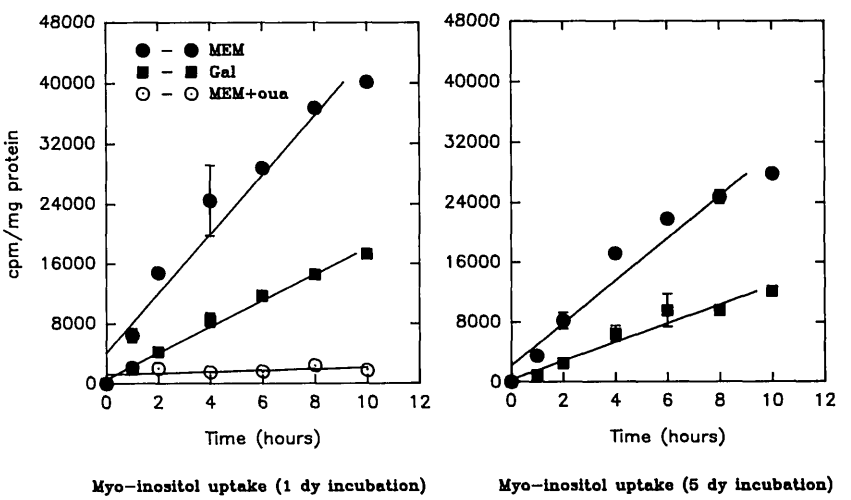

FIG. 1. Effect of galactose (Gal) on *myo*-inositol uptake in cultured bovine lens epithelial cells (BLECs). Confluent BLECs (25-cm² flasks) were maintained in physiological medium (Eagle's minimal essential medium [MEM]) or 40 mM Gal for 1- and 5-day exposure periods. At conclusion of preincubation period, corresponding fresh medium plus *myo*-[^{3}H]inositol (0.2 μCi/ml) was introduced to cultures, and time course of uptake of radiolabel was followed for 10 h. In separate experiment, 0.1 mM ouabain (oua) was included with physiological medium to inhibit Na^+-dependent entry of *myo*-[^{3}H]inositol. Replicate cultures were collected for each of designated times. Data points are means ± SE.

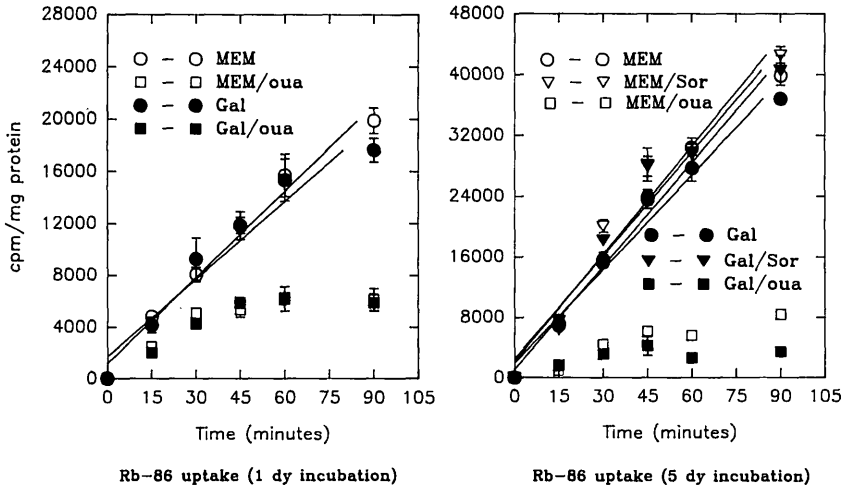

FIG. 2. Effect of galactose (Gal) on ^{86}Rb uptake in cultured bovine lens epithelial cells (BLECs). Confluent monolayers of BLECs were maintained in physiological medium (Eagle's minimal essential medium [MEM]) or 40 mM Gal for 1 day or MEM or Gal in presence and absence of sorbinil (Sor) for 5 days. At end of initial preincubation period, corresponding fresh medium plus 0.2 μCi/ml ^{86}Rb was introduced to cultures, and time course of uptake of radiolabel was followed for 90 min. In separate experiments, 0.1 mM ouabain (oua) was introduced into both physiological and Gal medium to inhibit Na^+-K^+-ATPase and entry of ^{86}Rb. Triplicate cultures were collected at designated times. Data points are means ± SE.

dium. Moreover, within 20 h of medium reversal to MEM alone, the galactose-withdrawn cells displayed a rate of *myo*-inositol uptake that was indistinguishable from the curve generated by the cells switched to physiological medium and sorbinil. That is, restoration of galactose-exposed cells to physiological medium, without the further addition of the aldose reductase inhibitor, mediated the normalization of *myo*-inositol uptake after only 20 h of recovery.

DISCUSSION

myo-Inositol uptake by lens epithelial cells in culture decreased after exposure to hypergalactosemic conditions. The uptake of *myo*-inositol occurs against a *myo*-inositol concentration gradient (20–30 mM intracellular) and is driven by the Na^+ gradient that is maintained by Na^+-K^+-ATPase pumping activity. Ouabain, an inhibitor of Na^+-K^+-ATPase, significantly depressed ^{86}Rb uptake and arrested *myo*-[^{3}H]inositol uptake, indicating that dissipation of the Na^+ gradient was sufficient to disrupt *myo*-inositol transport. Earlier studies by Kawaba et al. (10) demonstrated that rat lenses exposed to 30 mM galactose overnight prompted a

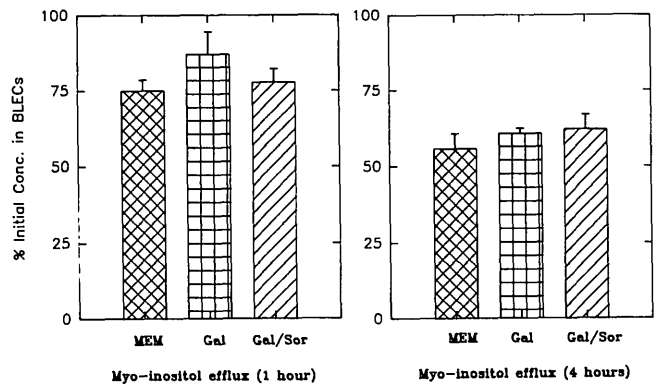

FIG. 3. Effect of galactose on *myo*-inositol efflux in cultured bovine lens epithelial cells (BLECs). Lens cultures were maintained in either physiological medium (Eagle's minimal essential medium [MEM]) or 40 mM galactose (Gal) in presence and absence of 10^{-4} M sorbinil (Sor) for 5 days before their incubation in 0.2 μCi/ml *myo*-[^{3}H]inositol for 4 h. Preloaded cells were switched to fresh medium without isotope, and triplicate cultures were collected after 1 and 4 h. Efflux of *myo*-inositol was expressed as percentage of radioactivity initially present in lens cells. Data are means ± SE.

lowering of *myo*-inositol–concentrating capability and a decrease in Na^+-K^+-ATPase pumping activity and Na^+-K^+-ATPase enzymatic activity. These studies, which were done with cultured lens cells, permitted us to chronologically identify the deficit in *myo*-inositol–concentrating ability as an early onset complication, which apparently preceded damage to Na^+-K^+-ATPase pumping activity as indicated by the attenuation of *myo*-[^{3}H]inositol uptake under conditions whereby ^{86}Rb uptake proceeded normally (Figs. 1 and 2). Indeed, the difference between this study and that of Kawaba et al. (10) can, in all likelihood, be accounted for by the degree of rapidity with which biochemical deficits had occurred in that system, thus making it impossible to observe a temporal distinction between the attenuation of *myo*-inositol uptake and a loss of Na^+-K^+-ATPase pumping activity. Alternatively, hypergalactosemia might deplete a discrete, small, galactose-sensitive intracellular *myo*-inositol pool that is essential for the maintenance of phosphatidylinositol-mediated processes, including Na^+-K^+-ATPase activity as suggested by Winegrad et al. (17). Although no change in total *myo*-inositol concentration was detected under the treatment conditions given, we cannot rule out the possibility that a small, independent, rapidly turning over *myo*-inositol pool might be important. Our observations neither support nor refute the existence of this mechanism in cultured BLECs. Furthermore, Greene et al. (4) have suggested that chronic hyperglycemia results in a substantial reduction in intracellular *myo*-inositol content, eventually causing a decrease in Na^+-K^+-ATPase activity. The current observation that the concentration of *myo*-inositol was not appreciably attenuated could account for the unaltered Na^+-K^+-ATPase activity in the lens epithelial culture system.

myo-Inositol concentration in the lens epithelium is an active mechanism and is linked to Na^+-K^+-ATPase through a Na^+-dependent process, as was first shown in the lens by Cotlier (18) and Varma et al. (19). The observation that ouabain addition significantly lowered ^{86}Rb uptake and arrested *myo*-[^{3}H]inositol uptake substantiates this point (Figs. 1 and 2). However, *myo*-inositol uptake, although linked to Na^+-K^+-ATPase via the requirement for a Na^+ gradient, is not strictly associated with the pump, because the galactose insult uncoupled the two events. Furthermore, Kawaba et al. (10) reported that the efflux of both *myo*-inositol and Rb

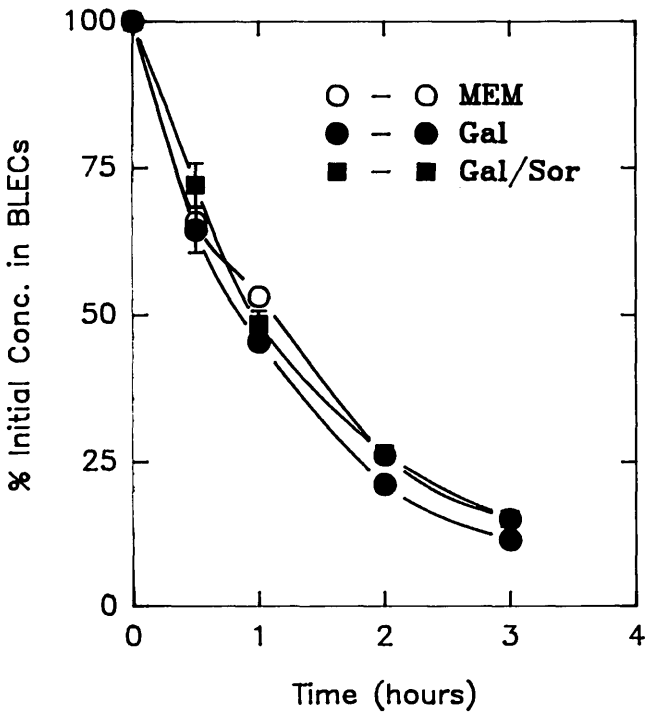

FIG. 4. Effect of galactose (Gal) on ^{86}Rb efflux in cultured bovine lens epithelial cells (BLECs). BLECs were treated as described in Fig. 3, except ^{86}Rb was substituted isotope. Preloaded cultures were switched to fresh medium without isotope, and triplicate cultures were collected at designated times over 3-h period. Data are expressed as percentage of radioactivity initially present in lens cells. Data points are means ± SE.

increased in the rat lens exposed to 30 mM galactose, and concluded that "the ability of the galactose-exposed lens to accumulate MI [*myo*-inositol] and Rb appears to be caused by changes in membrane permeability, leading to a rapid loss of these substances." Our data are not in agreement with that finding, because the rate of efflux of both *myo*-inositol and Rb was not observed to be appreciably different between the galactose-exposed and control cells (Figs. 4 and 5). Thus, the early onset loss of capability by the galactose-exposed cells to concentrate *myo*-inositol does not appear to be caused by a disruption of structural membrane integrity (under these experimental conditions), which otherwise would likely have led to increased membrane permeability and the rapid efflux of *myo*-inositol as described in other studies done with whole lenses (10).

These observations also suggest that the induced deficit in *myo*-inositol uptake may be directly attributed to the negative impact of galactose exposure on the *myo*-inositol transporter. In any event, our data make the possibility appear less likely that the increased efflux of *myo*-inositol and Rb resulting from sustained galactose exposure is the cause of the initial attenuation of *myo*-inositol accumulation. The mechanism of the galactose-induced inhibition of *myo*-inositol uptake is uncertain. However, it appears to involve the formation of galactitol via aldose reductase activity because sorbinil prevented the attendant reduction in *myo*-inositol–concentrating ability as elicited by galactose exposure. Because the galactitol content did not exceed 2 mM under our experimental conditions, the *myo*-inositol transporter appears to be sensitive to relatively low concentrations of polyols. Reports by Hammerman et al. (20) in renal brush border vesicles and Reshkin et al. (21) in isolated basolateral membrane vesicles of carnivorous and herbivorous teleosts have documented that the *myo*-inositol transporter is distinct from the D-glucose transporter. Similar to this study, the galactose-induced reduction in *myo*-inositol uptake in retinal pericytes was prevented with sorbinil pretreatment (22). Likewise, if galactose is removed from the incubation media after a continuous 5-day exposure, *myo*-inositol uptake normalizes within 20 h, suggesting that the damage to the *myo*-inositol transporter was reversible. The reversal of the *myo*-inositol uptake parallels a decrease in galactitol content, albeit a measurable amount of galactitol was present even though *myo*-inositol uptake normalized. This observation suggests that the *myo*-inositol transporter must be either extremely sensitive to the concentration of intracellular galactitol or indirectly subjected to modulation by the aldose reductase reaction. Previously, we have shown that the galactose-induced reduction in prostaglandin synthase (E.C. 1.14.99.1) activity and galactose-induced vacuole formation were reversed within 48 h when lens cells were placed in physiological media after a 5-day continuous exposure to 40 mM galactose (23). Collectively, these observations are consistent with the premise that hypergalactosemia produces reversible cellular damage that is suggestive of selective rather than generalized cellular sites of action.

The findings in this study are also consistent with the activation of the aldose reductase reaction or formation and accumulation of the product of the aldose reductase reaction

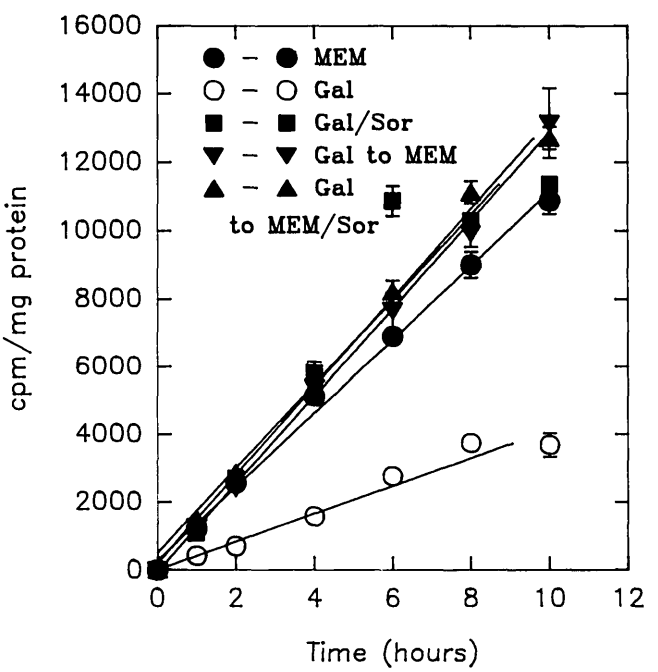

FIG. 5. Time course of *myo*-inositol uptake from cells cultured in physiological medium or galactose (Gal). Uptake of *myo*-[^{3}H]inositol was followed for 10 h in control cells (5.5 mM glucose, Eagle's minimal essential medium [MEM]), cells maintained in 40 mM Gal, Gal plus 10^{-4} M sorbinil (Gal/Sor) for 6 days. For medium-reversal studies, cultures were incubated for 5 days in Gal followed by 1 day of recovery in Gal-free medium (Gal to MEM) or Gal-free medium plus Sor (Gal to MEM/Sor). Data represent triplicate determinations from individual flasks. Data points are means ± SE.

being involved in the disruption of cellular functions described herein, rather than depletion of intracellular *myo*-inositol. Studies on diabetic cataract led to the "osmotic hypothesis," which states that accumulation of polyols directly promotes osmotic swelling, leading to diabetic complications (24,25). The best evidence to date that aldose reductase and polyols are involved in such complications of diabetes is the demonstration that aldose reductase inhibitors can prevent or delay the onset of complications (26,27). But any model that discusses the onset of diabetic complications via polyol accumulation must also take into account that many tissues may not accumulate galactitol to a concentration high enough to exert a direct osmotic effect. For instance, in one recent study, dogs fed a 30% galactose diet for 2 wk displayed no obvious symptoms of galactosemia or lens vacuole formation, whereas lens galactitol had increased to ~40 mM (28). In the same study, galactitol content in the sciatic nerve and retina was 100 times lower. In this study, BLECs maintained in 40 mM galactose for 6 days had a galactitol content that did not exceed 2 mM. It is not understood how polyol accumulation interferes with the *myo*-inositol transporter. Recently, Nakamura et al. (29) reported that cultured human retinal pigmented epithelial cells maintained in a high-glucose environment synthesized a chloroform-methanol–extractable product that comigrated with phosphatidylsorbitol by thin-layer chromatography. It is intriguing to speculate that cultured lens epithelium might be capable of synthesizing phosphatidylgalactitol. Indeed, the incorporation of phosphatidylgalactitol into the lipid or protein domain surrounding and in close juxtaposition to the *myo*-inositol transporter might be the underlying cause of the observed inhibition of *myo*-inositol uptake. In this regard, note that Jedziniak (30) recently demonstrated that the phosphatidylinositol-specific phospholipase C treatment of calf, rat, and human lens membrane preparations promoted the release of low-molecular-weight components from the membrane of all three lens types. She concluded that a complex assortment of polypeptides are attached to the membrane via a phosphatidylinositol bond. The incorrect insertion of phosphatidylgalactitol in place of phosphatidylinositol might negatively impact on the *myo*-inositol transporter and effectively depress *myo*-inositol uptake. This aberrant event might be able to occur with relatively little accumulation of intracellular polyol. Hence, requiring high concentrations of accumulated polyols to explain the sequence of events leading to diabetic complications may not be necessary. Studies are in progress to demonstrate the endogenous synthesis of phosphatidylgalactitol by cultured lens epithelium.

Previous studies from this laboratory have documented that cultured BLECs exposed to hypergalactosemic medium exhibit an accumulation of intracellular galactitol and a concomitant loss of glutathione (GSH) (23). In this study, we could not rule out the possibility that polyol accumulation and the loss of available GSH might both be partly responsible for the attenuation in ability to concentrate *myo*-inositol in the galactose-exposed cells. Alternatively, these two events may not be acting synergistically, such that their combined action or operation is not required to bring about the observed lowering of *myo*-inositol–concentrating ability in the galactose-exposed cells. We previously used the inhibitor of GSH biosynthesis L-buthionine sulfoximine to lower the intracellular pool of GSH from cells maintained in galactose-free physiological medium to a level below that routinely observed in galactose-incubated cells under conditions whereby no galactitol accumulation could occur (31). That approach permitted us to clarify whether the intracellular pool of GSH directly influenced prostaglandin endoperoxide synthase activity in cultured BLECs maintained in galactose-free physiological medium. L-Buthionine sulfoximine has been shown to specifically inhibit glutamate–cysteine ligase (E.C. 6.3.2.2; 32), and its use in those studies represented a tool by which the intracellular pool of GSH was artificially lowered without having to resort to galactose exposure. Experiments with a similar approach have been performed to clarify whether it is the loss of the intracellular pool of GSH or the accumulation of galactitol which elicits the attenuation of *myo*-inositol uptake in galactose-exposed cells (unpublished observations).

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