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Ambient glucose and aldose reductase-induced *myo*-inositol depletion modulate basal and carbachol-stimulated inositol phospholipid metabolism and diacylglycerol accumulation in human retinal pigment epithelial cells in culture

(diabetes/diabetic complications/sorbitol pathway/aldose reductase inhibitors/muscarinic cholinergic signal transduction)

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ABSTRACT **Physiological hyperglycemia has been speculated to alter phosphoinositide (PPI; inositol phospholipid) signal transduction in cells prone to diabetic complications by two separate mass-action mechanisms with antiparallel putative effects on diacylglycerol (DAG): (*i*) sorbitol-induced depletion of *myo*-inositol leads to diminished PPI synthesis and turnover and DAG release, and (*ii*) elevated glucose-derived DAG precursors enhance *de novo* DAG synthesis. Because the first mechanism is mediated by aldose reductase (AR2), which converts glucose to sorbitol, the effects of glucose on basal and stimulated PPI signaling were explored in lines of cultured human retinal pigment epithelial cells differing widely in their basal AR2 gene expression and enzymatic activity. The results suggest that the effects of glucose on PPI signaling vary inversely with the level of AR2 activity and parallel the extent of AR2-induced *myo*-inositol depletion.**

The hyperglycemia of diabetes has been speculated to alter phosphoinositide (PPI; inositol phospholipid) signal transduction, especially basal and agonist-stimulated intracellular diacylglycerol (DAG) mass, in cells prone to diabetic complications by one of two mechanisms. In the first, exemplified by peripheral nerve in experimental diabetes, glucose-induced aldose reductase (AR2)-mediated sorbitol accumulation induces reciprocal depletion of *myo*-inositol (MI), such that it becomes rate-limiting for critical components of PPI synthesis and arachidonoyl-DAG production that regulate Na^+, K^+-ATPase activity, leading to slow nerve conduction (1–3). This biochemical response pattern to glucose has been modeled in cultured human retinal pigment epithelial (RPE) cells (2, 4) expressing aberrantly high levels of the AR2 gene (5) and enzyme (6), which is responsible for conversion of glucose to sorbitol. However, the detailed interaction between glucose-induced MI depletion and inhibition of the phosphatidylinositol (PI) synthase reaction, for which MI and CDPdiglyceride (CDP-DG) function as cosubstrates, remains controversial (1, 7). Several independent lines of investigation suggest that this interaction may be quite complex, involving functionally discrete metabolic pools of MI, PPI, and/or PI synthase (1–3). In the second metabolic response pattern to glucose, exhibited *in vitro* by retinal *endothelial* and glomerular cells with relatively low AR2 activity (1, 8, 9), DAG content, glucose incorporation into DAG, and protein kinase C translocation from the cytosolic to the membrane fraction are all *increased* after exposure to hyperglycemic concentrations of glucose (10–12). This pattern has been ascribed to enhanced *de novo* DAG synthesis from glucose-derived precursors such as α-glycerophosphate and phosphatidic acid consequent to (13) or independent of (10–12) sorbitol pathway activation by glucose. This enhanced DAG synthesis has been speculated to exaggerate microvascular responses to growth factors and vasodilators in diabetes (10–13). The role of AR2 expression in determining which of these two response patterns to glucose predominates was explored in RPE cells, including a recently reported RPE cell line that constitutively overexpresses AR2 mRNA and enzyme (5, 6).

MATERIALS AND METHODS

Cell Culture. The Michigan Eye Bank supplied human postmortem eyes from which primary RPE cell lines were established as described (4). Experiments were performed on passage numbers 10–18 of RPE cell lines established from three separate patients (RPE 91, RPE 125, and RPE 45). RPE 91 constitutively overexpresses AR2 mRNA and enzyme (5, 6) and exhibits exaggerated sorbitol accumulation, MI depletion, and PI synthase inhibition when exposed to 20 mM glucose (2, 5, 6). Cells were cultured and exposed to experimental conditions for 7 days as described (2, 5, 6, 14).

Biochemical Measurements. MI and sorbitol were measured by GLC (4). For measurements of CDP-DG and ^{32}P incorporation into PI, cells grown in 10-cm dishes were labeled, respectively, with [5-^{3}H]cytidine and [^{32}P]orthophosphate, lipids were extracted, and the corresponding phospholipid band was separated by TLC and quantitated by liquid scintillation spectrometry (2). For PPI hydrolysis, cells grown in 3.5-cm dishes were labeled with [^{3}H]MI, rinsed, and exposed to different agonists in the presence of 10 mM lithium chloride; total inositol phosphates (IPs) were quantitated by liquid scintillation spectrometry (14). Cellular diglyceride (DG; i.e., DAG, alkylacylglycerol, and alkenylacylglycerol) mass was determined by its enzymatic conversion to [^{32}P]phosphatidic acid, purification by TLC, and quantitation by liquid scintillation spectrometry (15). Total and individual DAG molecular species were quantitated by purification of DG in the extracted lipids by TLC, benzoylation of the purified DG, purification of benzoylated DAGs

Abbreviations: AR2, aldose reductase; ARI, AR2 inhibitor; CDP-DG, CDPdiglyceride; DAG, diacylglycerol; DG, diglyceride; IP, inositol phosphate; MI, *myo*-inositol; PI, phosphatidylinositol; PPI, phosphoinositide (inositol phospholipid); RPE, retinal pigment epithelial.

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by a second TLC and by normal-phase HPLC, and separation of the individual molecular species by reversed-phase HPLC. The peaks were identified from the retention times obtained for a mixture of seven known DAGs analyzed under identical conditions. The area of the peaks was integrated in a computer attached to the HPLC (Maxima; Waters), and the values were converted to picomoles per milligram of total protein (16).

RESULTS

Effect of Glucose and an AR2 Inhibitor (ARI) on Sorbitol and MI Contents in "High AR2" RPE 91 and "Low AR2" RPE 125 and RPE 45 Cells. Exposure of RPE 91 to 20 mM glucose for 7 days increased sorbitol content from 8.2 ± 0.9 to 98.4 ± 29.5 nmol/mg of protein ($P \leq 0.05$; $n = 3$); in RPE 125 and RPE 45, sorbitol was undetectable under these conditions. Sorbitol accumulation in RPE 91 was associated with reciprocal depletion of MI. Twenty millimolar glucose failed to alter MI in RPE 125 and RPE 45 (Fig. 1). In RPE 91 exposed to 20 mM glucose, ARI (10 μM sorbinil) decreased sorbitol to undetectable levels and reversed the reduction in MI. Sorbinil had no effect on MI in RPE 125 and RPE 45 (Fig. 1) (6).

Effect of Glucose and ARI on Basal and Stimulated PI Synthase in High and Low AR2 RPE Cells. In 5 mM glucose, a maximally stimulating concentration of carbachol (14) produced no significant change in [^{3}H]CDP-DG in RPE 91 cells (Fig. 2*A*), despite a 2- to 3-fold increase in ^{32}P incorporation into PI (Fig. 3). These findings are consistent with an increased rate of resynthesis of PI following carbachol-stimulated PPI hydrolysis (14) with either PPI-derived CDP-DG rather than MI being the rate-limiting substrate or carbachol coordinately stimulating phospholipase C and PI synthase. Conditioning RPE 91 in 20 mM glucose for 7 days reproduced the previously reported doubling of [^{3}H]CDP-DG and the 60% decrease in basal ^{32}P incorporation into PI, attributable to inhibition of basal PI synthase by MI depletion (Figs. 2*A* and 3) (2). The increase in basal [^{3}H]CDP-DG in 20 mM glucose was not further exaggerated by carbachol stimulation, despite a 250% increase in ^{32}P incorporation into PI. Thus, when expressed as a percentage of the basal incorporation, the increment in ^{32}P incorporation into PI produced by carbachol was not different in RPE 91 in 5 vs. 20 mM glucose (337% ± 16% vs. 317% ± 15%; *P*, not significant), despite identical reduction in both basal and stimulated incorporation in the latter condition. These observations are consistent with the hypothesis that carbachol-stimulated PI synthesis may partly bypass or reverse the inhibition of the PI synthase reaction caused by MI depletion. In contrast to RPE 91, RPE 45 exhibited no consistent alteration after 20 mM glucose exposure for 7 days in basal [^{3}H]CDP-DG (Fig. 2*B*) or in carbachol stimulation of ^{32}P incorporation into PI, phosphatidylinositol 4-phosphate, or phosphatidylinositol 4,5-bisphosphate (respectively, 303% ± 27%, 111% ± 9%, and 103% ± 10% in 5 mM glucose vs. 324% ± 47%, 154% ± 10%, and 132% ± 14% in 20 mM glucose; *P* not significant). Similar results were obtained for RPE 125 cells. The lack of [^{3}H]CDP-DG accumulation in RPE 125 and RPE 45 exposed to 20 mM

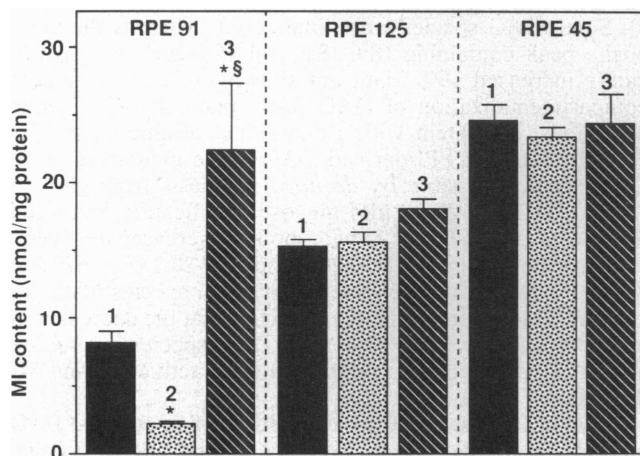

FIG. 1. Effect of 5 and 20 mM glucose and ARI on MI content in RPE cells with high (RPE 91) and low (RPE 125 and RPE 45) AR2 expression. Cells were incubated for 1 week in 5 (bar 1) or 20 (bar 2) mM glucose or in 20 mM glucose plus 10 μM sorbinil (bar 3). MI content was determined by GLC as described in *Materials and Methods* ($n = 3$; *, $P < 0.05$ compared to 5 mM glucose; §, $P < 0.05$ compared to 20 mM glucose).

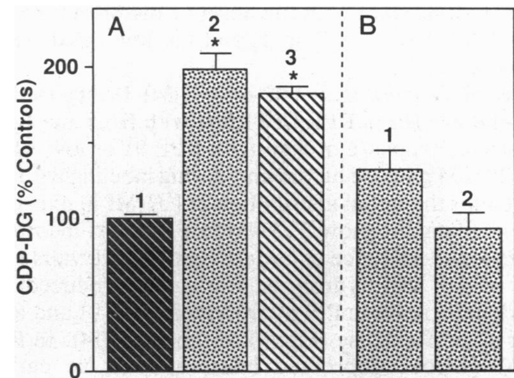

FIG. 2. Effect of 5 and 20 mM glucose and carbachol on CDP-DG levels in RPE cells with high (*A*) and low (*B*) AR2 expression. Cells were exposed to 5 or 20 mM glucose for 1 week, and CDP-DG levels were determined as described in *Materials and Methods*. Data are expressed as a percentage of the relevant 5 mM glucose control ($n = 4$; *, $P < 0.05$ compared to respective controls in the presence of 5 mM glucose). (*A*) RPE 91 cells. Bar 1, cells incubated in 5 mM glucose and stimulated with 10 mM carbachol for 1 h; bar 2, cells incubated in 20 mM glucose; bar 3, cells incubated in 20 mM glucose and stimulated with carbachol. (*B*) RPE 125 (bar 1) or RPE 45 (bar 2) cells were incubated in 20 mM glucose.

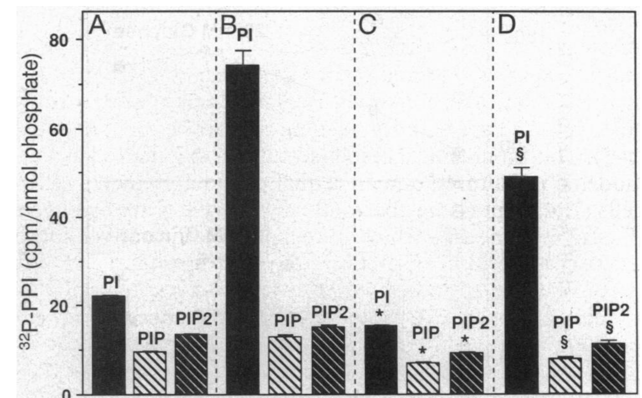

FIG. 3. Effect of 5 (*A* and *B*) and 20 mM (*C* and *D*) glucose on basal (*A* and *C*) and carbachol-stimulated (*B* and *D*) ^{32}P incorporation into PPI in RPE cells with high intrinsic AR2 expression. RPE 91 cells were exposed to 5 or 20 mM glucose for 1 week, labeled with [^{32}P]orthophophate for 2 h in phosphate-free medium, and in some cases stimulated with 10 mM carbachol during the last 1 h of the labeling period, and [^{32}P]PPIs were determined as described in *Materials and Methods*. The values represent the mean ± SE from four different replicate samples of PPI from a representative experiment (*, $P < 0.05$ compared to 5 mM glucose; §, $P < 0.05$ compared to 20 mM glucose). Identical results were obtained for two other independent experiments. PIP, phosphatidylinositol 4-phosphate; PIP2, phosphatidylinositol 4,5-bisphosphate.

glucose is consistent with the absence of sorbitol accumulation and MI depletion (Fig. 1) and the low basal AR2 gene expression (5, 6).

Effect of Glucose and ARI on [^{3}H]MI Incorporation into Water-Soluble IPs in RPE Cell Lines with High and Low AR2 Expression. Exposure of high AR2 RPE 91 or low AR2 RPE 125 to 20 mM glucose before and during labeling with [^{3}H]MI did not alter the initial distribution of [^{3}H]MI in phospholipid fractions (data not shown). Therefore, [^{3}H]MI incorporation into IPs in the presence of lithium represents a valid estimate of the rate of PPI hydrolysis. Carbachol produced a dose-dependent increase in PPI hydrolysis in RPE 91 and RPE 125 in 5 or 20 mM glucose (Fig. 4) as reported (14). In RPE 91, 20 mM glucose depressed basal as well as carbachol-stimulated PPI hydrolysis at all concentrations of agonist tested (data not shown). Sorbinil completely corrected the reduction in PPI hydrolysis induced by 20 mM glucose in RPE 91 (Fig. 5). These data suggest that in this high AR2 cell line (5, 6), glucose depresses PPI hydrolysis by an AR2-mediated process associated with MI depletion and inhibition of PI synthase despite the lack of any consistent reduction in PPI chemical mass (2). Exposure of RPE 125 to 20 mM glucose marginally diminished basal PPI hydrolysis but paradoxically enhanced carbachol-stimulated PPI hydrolysis (Fig. 4*B*). A similar overall response pattern was exhibited by low AR2 RPE 45 (data not shown). Thus hyperglycemic concentrations of glucose appear to induce opposite effects on carbachol-stimulated PPI hydrolysis in cells with low and high basal AR2 expression.

Effect of Glucose on Basal Levels of Total DAG and DAG Molecular Species in RPE cells with High or Low AR2 Expression. Despite the marked sorbitol accumulation and MI depletion in RPE 91 exposed to 20 mM glucose for 7 days, total DG content measured enzymatically was unaffected by glucose exposure (1.47 ± 0.09 pmol/nmol of phosphate in 5 mM glucose and 1.44 ± 0.11 pmol/nmol of phosphate in 20 mM glucose; $n = 3$; P not significant). Because these enzymatic mass analyses detect all DGs, including but not limited to DAG, and because the molecular species of DAG derived *de novo* from glucose differ from those released by PPI hydrolysis (17), the distribution and concentration of individual DAG molecular species (16) were determined in unstimulated and carbachol-stimulated high AR2 RPE 91 and low AR2 RPE 45 in 5 or 20 mM glucose for 7 days. The total DAG levels (sum of the separated species) were marginally lower in RPE 91 vs. RPE 45 under unstimulated conditions in 5 mM glucose: 156.3 ± 29.3 ($n = 6$) vs. 230.4 ± 41.4 ($n = 3$) pmol/mg of protein (P not significant). The 18:0-20:4 species (Fig. 6*A*), which has been previously shown to be the major DAG species released by PPI hydrolysis (18), coeluted with 16:0-20:3-DAG (16) and together constituted ≈2% of total DAG in both cell lines. Trivial differences in the distributions of other molecular species were seen between the two cell lines (data not shown).

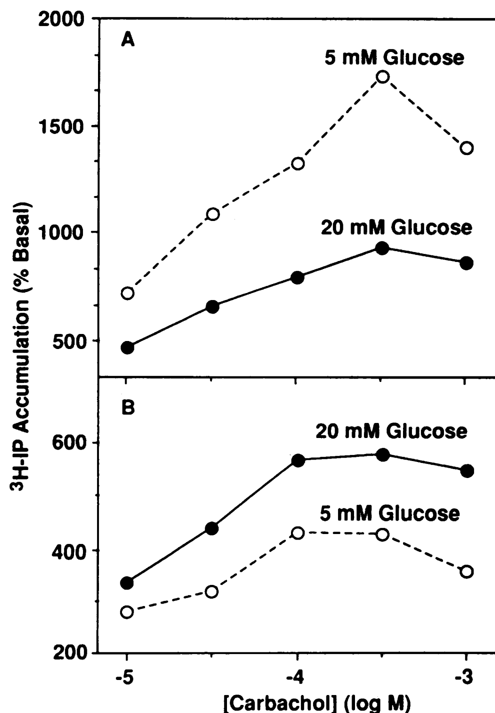

FIG. 4. Effect of glucose on carbachol stimulation of [^{3}H]MI incorporation into IPs in RPE cells with high (*A*) and low (*B*) AR2 expression. Cells were exposed to either 5 or 20 mM glucose for 1 week and labeled with [^{3}H]MI for 24 h. The cells were then stimulated for 1 h with various concentrations of carbachol, and [^{3}H]IP was determined as described in *Materials and Methods*. The values are representative of either four (*A*) or three (*B*) independent experiments.

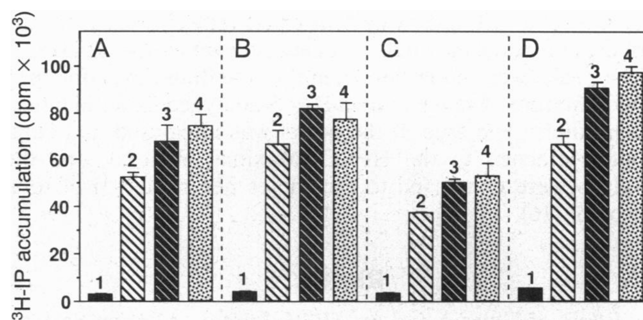

FIG. 5. Effect of ARI on glucose-mediated perturbation of carbachol-stimulated [^{3}H]MI incorporation into IPs in RPE cells with high AR2 expression. RPE 91 cells were exposed for 1 week to 5 (*A* and *B*) or 20 mM (*C* and *D*) glucose in the presence (*B* and *D*) or absence (*A* and *C*) of 10 μM sorbinil. The cells were then stimulated with 10^{-5} (bar 1), 3.16×10^{-5} (bar 2), 3.16×10^{-4} (bar 3), or 10^{-3} (bar 4) M carbachol and analyzed for [^{3}H]IP as described for Fig. 4. The data represent the mean ± SD of triplicate cell samples. Identical results were obtained for two other independent experiments.

Exposure of RPE 91 to 20 mM glucose for 7 days marginally lowered total DAG, but several DAG species including those most likely derived from PPI turnover (e.g., 18:0-20:4 and 16:0-20:3) were reduced by 20–30% (Fig. 6*A*, bars 1 and 4). Some DAG species were unaffected, whereas the composite peak containing 18:0-18:1 and 16:0-20:1 was significantly increased 19% (data not shown), indicating distinct compartmentalization of DAG pools under hyperglycemic conditions, consistent with a dual effect of glucose to specifically diminish PPI-derived DAG while increasing other DAG species possibly by *de novo* synthesis from glucose (11–13). In RPE 45, 20 mM glucose significantly increased total DAG (Fig. 6*B*, bar 1) with modest increases in several DAG species excluding 18:0-20:4 and 16:0-20:1 (Fig. 6*B*, bar 4) and no detectable decrease in any DAG species (data not shown). This is consistent with the view that the decreases in total DAG and certain PPI-derived DAG species by glucose in RPE 91 resulted from polyol pathway activation and MI depletion.

Effect of Glucose and Carbachol Stimulation on Total DAG and DAG Molecular Species in High and Low AR2 RPE Cells. The time-dependent effects of 10 mM carbachol on various DAG molecular species were identical in RPE 91 and RPE 45, reaching maximal at 5–10 min (data not shown); the latter time point was employed in all subsequent experiments. Carbachol induced a 3-fold increase in total DAG in RPE 91 conditioned at 5 or 20 mM glucose (Fig. 6*A*, bars 2 and 3). In RPE 45, the 47% higher basal total DAG level increased only

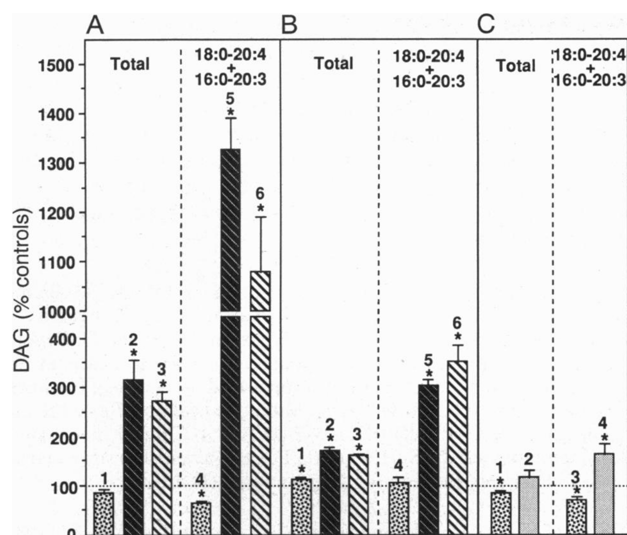

FIG. 6. Effect of carbachol stimulation and MI supplementation on glucose-mediated perturbation of DAG content and 18:0-20:4-DAG plus 16:0-20:3-DAG. High AR2 RPE 91 (*A*) or low AR2 RPE 45 (*B*) were exposed for 1 week to 5 or 20 mM glucose and stimulated with carbachol for 10 min, and the individual DAG species were quantitated by HPLC. Total DAG was calculated as the sum of individual species. Data are expressed as the percentage of the relevant 5 mM glucose control (dotted line). Bars 1 and 4, cells incubated in 20 mM glucose; bars 2 and 5, cells incubated in 5 mM glucose and then stimulated with carbachol; bars 3 and 6, cells incubated in 20 mM glucose and then stimulated with carbachol. (*C*) RPE 91 were exposed for 1 week to 5 mM glucose, 20 mM glucose (bar 1), or 20 mM glucose + 500 μM MI (bar 2), and the individual DAG species were quantitated as described above. Data are expressed as the percentage of the 5 mM glucose control (dotted line).

0.6- to 0.7-fold with carbachol (Fig. 6*B*, bars 2 and 3), but the molecular pattern of stimulation was similar in the two cell lines, with maximal stimulation occurring in the 18:0-20:4 and 16:0-20:3 species, which increased 10- and 3-fold, respectively, in RPE 91 and RPE 45 (Fig. 6 *A* and *B*, bars 5 and 6).

In RPE 91, 20 mM glucose lowered basal levels of 18:0-20:4 and 16:0-20:3 DAG (Fig. 6*A*, bar 1) as well as the two composite peaks containing 16:1-18:1-, 18:1-18:2-, 18:1-22:4-, and 18:1-20:3-DAG and 16:0-18:1-, 18:0-18:2-, 18:0-22:4-, and 18:0-20:3-DAG. The percent stimulation by carbachol for total DAG and 18:0-20:4- and 16:0-20:3-DAG was virtually identical in 5 or 20 mM glucose (Fig. 6*A*, bars 2 and 3 and bars 5 and 6), as well as for all other DAG groups (data not shown). Accordingly, the absolute DAG levels attained by these species were also lower after carbachol stimulation in proportion to their lower basal levels (data not shown). Thus, exposure of high AR2 RPE cells to 20 mM glucose reduced the levels of the more unsaturated DAG molecular species (presumably derived from PPI) to a similar degree in both the basal and carbachol-stimulated state, paralleling the effects of 20 mM glucose on PI synthesis and PPI turnover. In low AR2 RPE 45, 20 mM glucose marginally increased total but not 18:0-20:4- and 16:0-20:3-DAG (Fig. 6*B*, bars 1 and 4) in the basal state without significantly affecting the percent stimulation or maximal levels after carbachol of any DAG molecular species (Fig. 6*B*, bars 2 and 3 and bars 5 and 6).

Effect of Glucose, MI Supplementation, and ARI on Basal and Carbachol-Induced DAG Molecular Species in High AR2 RPE Cells. The supposition that AR2-induced MI depletion mediated the effects of glucose on DAG content and molecular species in high AR2 RPE cells was explored by studying the effect of MI supplementation (500 μM) exceeding that necessary to raise intracellular MI and overcome the inhibitory effect of 20 mM glucose on PI synthesis in RPE 91 (2). Again, 20 mM glucose depleted total and 18:0-20:4- and 16:0-20:3-DAG (as well as other related groups), but MI supplementation completely normalized total DAG to a level indistinguishable from that of the control in 5 mM glucose alone (Fig. 6*C*, bars 1 and 2) or in 5 mM glucose plus 500 μM MI (data not shown). MI actually overcorrected the reduction in 18:0-20:4 and 16:0-20:3 (Fig. 6*C*, bars 3 and 4). Qualitatively similar results were obtained with 10 μM sorbinil under otherwise identical conditions (data not shown). Thus, MI supplementation or an ARI specifically reversed the perturbations of DAG associated with MI depletion in 20 mM glucose in RPE 91 (2), supporting the view that MI depletion underlies the glucose-induced perturbations of DAG in high AR2 cells.

Effect of Glucose-Induced Hyperosmotic AR2 Gene Induction and MI Depletion on DAG Content in Low AR2 Cells. Hyperosmotic (590 milliosmoles/kg) stress induces AR2 mRNA and enzyme in low AR2 cells to levels of high AR2 RPE 91 (5, 6), although the rise in enzymatic activity is delayed (6). As anticipated by the compatible osmolyte hypothesis of Burg, and as recently reported (6), exposure of low AR2 RPE 45 to 300 mM glucose produced an initial compensatory hyperosmotic accumulation of MI, followed by AR2 gene induction, sorbitol accumulation, and ARI-sensitive MI depletion approximating that of high AR2 RPE cells exposed to 20 mM glucose alone for 7 days (6) (data not shown). To explore the link between AR2 expression, MI depletion, and DAG metabolism, DAG levels were quantitated in RPE 45 exposed to 300 mM glucose for 7 days. Total DAG was decreased by 63.1% ± 9.3% vs. cells exposed to 5 mM glucose ($P < 0.05$) with the decrease involving all DAG species except 18:0-22:5, 17:0-18:1, and 16:0-18:0 (data not shown). Thus in cells with constitutively low AR2 expression, glucose-induced DAG depletion again parallels MI depletion after the AR2 gene is induced by hyperosmotic stress.

DISCUSSION

This communication compares the effect of glucose on MI content and basal and agonist-stimulated PI synthase inhibition, PI synthesis, PPI turnover, and DAG generation in RPE cells with high and with low AR2 activity. Twenty millimolar glucose depleted MI in high AR2 RPE 91 and inhibited basal PI synthase leading to CDP-DG accumulation and decreased ^{32}P incorporation into PPI (2). Basal PPI turnover, as measured by [^{3}H]MI incorporation into IP, and the basal DAG levels were also reduced by 20 mM glucose. Induction of increased AR2 expression and activity and attendant MI depletion in low AR2 RPE 45 by hyperosmotic hyperglycemic stress (300 mM glucose) resulted in similar depletion of MI and DAG. When AR2 activity was low, or when glucose-induced MI depletion was prevented in high AR2 RPE 91 by MI supplementation or ARI, 20 mM glucose tended to increase rather than decrease basal PPI turnover and DAG, consistent with the proposed enhanced *de novo* synthesis of PPI precursors by glucose (11–13, 17).

In high AR2 RPE 91, 20 mM glucose and the attendant MI depletion in general decreased basal and carbachol-stimulated PPI metabolism to the same relative degree. (Twenty millimolar glucose attenuated carbachol-stimulated PPI hydrolysis to a somewhat greater extent than basal hydrolysis, suggesting that [^{3}H]MI incorporation into IPs may be a more sensitive indicator of inhibition of stimulated PPI turnover.) These glucose-induced changes in basal and carbachol-stimulated ^{32}P incorporation into PPI and PPI hydrolysis in RPE 91 with high AR2 expression were partially or completely prevented by ARI or MI supplementation. Thus glucose impairs basal as well as stimulated accumulation of PPI-derived DAG in high AR2 cells through polyol

pathway activation, MI depletion, PI synthase inhibition, and lowered basal and stimulated PPI turnover (1). The lack of exaggerated glucose-induced CDP-DG accumulation and PI synthase inhibition after carbachol stimulation suggests that either agonist-induced phospholipase C activation preferentially hydrolyzes glucose-insensitive (or less glucose sensitive) PPI pools or that M_3 muscarinic agonists coordinately increase both phospholipase C and PI synthase activities, the latter either directly or via compartmentalized release of MI from PPI.

This persistent defect in PPI turnover and distribution of DAG molecular species after carbachol stimulation exemplifies impaired PPI-mediated signal transduction in glucose-exposed, MI-depleted RPE cells with high AR2 activity. A similar biochemical pattern has been described in sciatic nerve from the streptozotocin-induced diabetic rat, where carbachol-stimulated PPI turnover and arachidonoyl-DAG are reduced (3), and in cultured calf retinal pericytes, where endothelin 1-induced DAG accumulation and protein kinase C activation are blunted after glucose exposure (12). Thus, RPE 91 may appropriately model cells with high AR2 activity that are susceptible to diabetic complications. Whether other PPI-dependent signal transduction pathways such as intracellular calcium mobilization or PI-3-kinase are also affected by glucose-induced MI depletion in such cells with high AR2 activity remains to be explored in RPE 91 and other appropriate models.

These observations argue strongly that the directionality of glucose-induced perturbation of basal and stimulated PPI metabolism reflects AR2 expression and AR2-dependent MI depletion rather than other intrinsic characteristics of high and low AR2 cell lines. According to this construct, the degree of AR2 expression and/or activity would determine which of the two most frequently cited effects of glucose on PPI- or DAG-mediated signal transduction predominates: AR2-mediated MI depletion, PI synthase inhibition, and reduced arachidonyl-DAG (1, 3) on one hand; or a more generalized increase in *de novo* DAG synthesis by AR2-independent or AR2-dependent mechanisms on the other (11, 12, 19). [*In vitro* studies suggest that protein kinase C is activated equally by DAG species with various saturated or unsaturated fatty acyl chains (20). However *in vivo* data suggest that DAGs derived from different membrane lipids and/or various protein kinase C subspecies may be highly compartmentalized intracellularly (21).] Thus disparate effects of glucose on DAG in high and low AR2 conditions may have quantitatively and/or qualitatively distinct implications for DAG-mediated signaling (21).

The intriguing possibility that glucose could have opposite effects on DAG in cells with high or low AR2 activity warrants further investigation, especially in view of the cell-specific localization of AR2 in cellularly heterogeneous tissues that exhibit diabetic complications (1, 7, 8). Discordant effects of glucose on DAG levels and molecular species in retinal pericytes (11) and endothelial cells (9) could produce simultaneous but diametrically opposite or at least divergent (21) effects on growth factor responsiveness and the cell cycle, promoting the simultaneous pericyte loss and endothelial cell replication characteristic of early diabetic retinopathy. Similarly, AR2-related reductions in arachidonoyl-DAG and related molecular species in diabetic nerve could also explain some of the reported beneficial effects of dietary essential fatty acid supplementation (22, 23) or prostaglandin analogs (24) on nerve function in the streptozotocin-induced diabetic rat. Variations in AR2 gene expression or activity over time and between species producing antiparallel effects on MI and DAG could also partially address the so-called "*myo*-inositol paradox" as described by Matschinsky and coworkers (7). Finally, ARI treatment may theoretically convert the biochemical response to hyperglycemia from a pattern of reduced PPI-derived DAG to one of increased DAG synthesis in a tissue-specific manner depending upon intrinsic level AR2 activity, the degree of AR2 inhibition, and the capacity for *de novo* synthesis of PPI precursors from glucose.

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