Regulators for Blood Glucose Level Affect Gene Expression of Aquaporin 3

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Aquaporin 3 (AQP3), a membrane protein, is known to permeabilize water and other small molecules such as glycerol and urea and is localized in the bowel, skin, kidney, and erythrocytes. Since glycerol is a nutrient and serves as a source material in glycolytic metabolism, absorption of glycerol in the gastrointestinal tract may be under some control. Therefore we first investigated whether insulin regulating the glycolytic pathway took part in glycerol transport through AQP3 in the gastrointestinal tract and found that insulin significantly suppressed mRNA and protein expressions of AQP3 in Caco-2 cells. The antidiabetic drugs troglitazone and tolbutamide were also observed to suppress significantly AQP3 expression, but the biguanides metformin and buformin did not induce such suppression. Epinephrine was found to increase expression of AQP3, although glucagon showed no change of expression. Wortmannin and rapamycin were demonstrated to deactivate suppression of AQP3 expression by insulin and troglitazone, suggesting that the signal transducers, phosphoinositide 3 kinase (PI3K) and the mammalian target of rapamycin (mTOR), are involved in the signal pathway for regulating transcription of AQP3.

Key words aquaporin 3; insulin; antidiabetic; epinephrine; phosphoinositide 3 kinase

Materials

Recombinant human insulin, buformin hydrochloride, calphostin C, and H89 were purchased from Wako Pure Chemicals, Ltd. (Osaka, Japan). Epinephrine hydrochloride and wortmannin were gained from Sigma-Aldrich Co. (St. Louis, U.S.A.). Tolbutamide, rapamycin, glucagon, and metformin hydrochloride were obtained from Nacalai Tesque, Inc. (Kyoto, Japan), Merck Biosciences-Calbiochem, Inc. (Darmstadt, Germany), Peptide Institute, Inc. (Osaka, Japan), and ICN Biomedicals Inc. (Irvine, U.S.A.), respectively. TRIzol and SuperScript III reverse transcriptase and Oligotex-dT30 (Super) were obtained from Invitrogen Corp. (Carlsbad, CA, U.S.A.) and Takara Bio Inc. (Osaka, Japan), respectively. DNA-manipulating enzymes were gained from Promega Corp. (Madison, WI, Wisconsin, U.S.A.).

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RESULTS

Effects of Hypoglycemic Agents on AQP3 Expression
mRNA and protein expressions of AQP3 in Caco-2 cells treated with approximately physiological concentration of insulin were investigated by real-time RT-PCR. mRNA expression of AQP3 was observed to decrease significantly after treatment of the cells with insulin at concentrations of 10—100 nM for 12 h and at a concentration of 100 nM for 12 and 24 h (Figs. 1A, B). Protein expression of AQP3 was also observed to decrease 48 h after 1 μM insulin treatment (Fig. 1C).

AQP3 expression in cells treated with hypoglycemic reagents troglitazone, tolbutamide, and biguanides was also examined. Troglitazone and tolbutamide were demonstrated to decrease mRNA expression of AQP3 at concentrations of 10—100 μM for both drugs with 12 h of treatment (Figs. 2A—D). The concentration of 10 μM tolbutamide was within the concentration range used clinically. On the other hand biguanides metformin and buformin did not affect AQP3 expression within the concentrations indicated in Figs. 2E and F even though the concentrations of biguanides used in this experiment were much higher than those clinically used.

Effects of Hyperglycemic Hormones on AQP3 Expression mRNA expression of AQP3 in Caco-2 cells treated with approximately physiological concentrations of epinephrine and glucagon was measured. Epinephrine at concentrations of 1—100 nM for 12 and 24 h caused AQP3 expression to increase, whereas the expression was unaffected by treatment with glucagon (Fig. 3).

Effects of Inhibitors of Signal Transduction Pathway on AQP3 Expression To clarify the mechanism of suppression of AQP3 expression by treatment with insulin and troglitazone, signal transduction pathways regulating AQP3 expression were studied using inhibitors for the pathways. Wortmannin and rapamycin, inhibitors for phosphoinositide 3 kinase (PI3K) and mammalian target of rapamycin (mTOR), respectively, were found to recover AQP3 expression decreased by insulin and troglitazone (Figs. 4A, B, D, E). Glycogen synthase kinase-3 (GSK-3) did not affect expression of AQP3 and recovered the expression decreased by insulin and troglitazone (Figs. 4C, F).

It was also examined whether protein kinases A and C participated in the increase of AQP3 expression by epinephrine. Although calphostin C, an inhibitor for protein kinase C, suppressed increase of expression, the expression was unaffected by H89, an inhibitor for protein kinase A (Fig. 5).
agents for controlling blood glucose level on the expression of AQP3 mRNA in Table 1 and show the proposed pathways of the AQP3 expression regulated by insulin and epinephrine receptors signaling in Chart 1.

**DISCUSSION**

Insulin is well known to regulate transcription of various genes encoding enzymes in the metabolic pathways of glycolysis, gluconeogenesis, glycogen synthesis, and fatty acid synthesis. We found that insulin significantly suppressed mRNA and protein expression of AQP3 in Caco-2 cells in this study. Although the hypoglycemic agents troglitazone and tolbutamide were also demonstrated to suppress AQP3 mRNA expression, biguanides metformin and buformin did not affect expression. Furthermore, treatment with epinephrine, a hyperglycemic hormone, resulted in increase of the AQP3 mRNA expression, but glucagons, exhibiting the same effect as epinephrine, did not elicit increased expression. Thus AQP3 may be expected to have direct or indirect involvement in the control of blood glucose level because of the finding that mRNA and protein expression of AQP3 is regulated by hormones and medicines that control blood glucose level. Since AQP3 has been reported to pass glycerol as well as water through plasma membranes, it is likely that AQP3 in the gastrointestinal tract participates in absorption of glycerol in ingested nutrition. We could understand more clearly how AQP3 is involved in control of blood glucose level if we can measure how much glycerol as a source in glycolytic metabolism is carried by AQP3 in the gastrointestinal tract.

Signal transduction pathways of insulin have been studied in depth for enzymes involved in glycolytic metabolism, transporters, and proteins related to cell growth. Glucose 6-phosphate dehydrogenase (G6PD) was reported to increase its enzymic activity and mRNA expression by insulin both in vitro and in vivo. Wagle et al. suggested that the presence of PI3K and mTOR was necessary in the signal transduction pathway of G6PD expression by insulin from results of studies with various inhibitors for the signal pathways PD98059, wortmannin, LY294002, and rapamycin. Glucose 6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) were also reported to undergo increased expression by insulin treatment and this overexpression was deactivated by simultaneous addition of wortmannin. PI3K was demonstrated to participate in the signal pathway from insulin that controls expression of not only glycolytic enzymes but also glucose transporter 4, insulin-like growth factor-binding protein-1 (IGFBP-1), and Na+/Pi-cotransporter-
1.15—17 Lithium chloride, an inhibitor of GSK-3, was shown to restore expression of IGFBP-1 suppressed by insulin, suggesting that GSK-3 is necessary for regulating transcription of IGFBP-1.18 Suppression of AQP3 transcription by insulin was found recovered by simultaneous addition of wortmannin and rapamycin, inhibitors for PI3K and mTOR, respectively, but no change was observed by lithium chloride in this study, suggesting that the signal transduction pathway from insulin for the expression of AQP3 includes PI3K and mTOR but not GSK-3.

Tumor necrosis factor-α (TNF-α) was reported to induce insulin resistance in skeletal muscles by continuous i.v. injection to rats, and is generally believed a causative agent inducing this resistance.19 TNF-α causes increase of cellular ceramides and activation of PKC through activation of sphingomyelinase, and this activation causes phosphorylation of serine residues in insulin receptor substrate-1 (IRS-1).20 Therefore tyrosine kinase activity of the insulin receptor is inhibited and insulin resistance appears to occur by stopping signal transduction from insulin. Since thiazolidinedione derivatives pioglitazone and troglitazone are ligands for peroxi-
some proliferator activated receptor-\(\gamma\) (PPAR-\(\gamma\)), the action mechanism of the derivatives has been proposed to restore the impaired function of IRS-1 by normalizing production of TNF-\(\alpha\). Thus the derivatives improve signal transduction downstream of IRS-1 and the effect of insulin increase in target organs. Troglitazone was reported to suppress mRNA expressions of G6Pase and PEPCK and induce expression of GLUT4 in adipose cells. mRNA expression of AQP3 in Caco-2 cells was found to decrease by treatment with troglitazone in this study. The suppressed expression was improved by simultaneous addition of wortmannin and rapamycin, suggesting that the signaling pathway from troglitazone partly shares the pathway from insulin downstream of IRS-1.

Epinephrine was observed to increase mRNA expression of AQP3. This increase was suppressed by simultaneous treatment with calphostin C, an inhibitor for protein kinase C (PKC), but not with H89, an inhibitor for protein kinase A (PKA), suggesting that the signaling pathway goes through PKC. Itoh et al. reported that vasoactive internal polypeptide (VIP) induced increase of AQP3 expression in a human colonic epithelial cell line and that this phenomenon occurred through PKA. This may be explained by differences of the signal pathway between insulin and VIP in spite of involving increased AQP3 expression. Tolbutamide was observed to decrease mRNA expression of AQP3 and this may imply the possibility that the suppression mechanism of AQP3 expression by tolbutamide is partially similar to that of insulin. On the other hand, metformin and buformin did not suppress AQP3 expression, suggesting the possibility that the signaling pathway of the derivatives has been proposed to restore the impaired function of IRS-1 by normalizing production of TNF-\(\alpha\). Thus the derivatives improve signal transduction downstream of IRS-1 and the effect of insulin increase in target organs. Troglitazone was reported to suppress mRNA expressions of G6Pase and PEPCK and induce expression of GLUT4 in adipose cells. mRNA expression of AQP3 in Caco-2 cells was found to decrease by treatment with troglitazone in this study. The suppressed expression was improved by simultaneous addition of wortmannin and rapamycin, suggesting that the signaling pathway from troglitazone partly shares the pathway from insulin downstream of IRS-1.

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The signal pathway from insulin and troglitazone for regulation of AQP3 expression is predicted to pass through PI3K and mTOR in this study. Kishida et al. observed suppression of AQP7 expression by treatment with insulin in adipose cells and investigated a promoter region for the gene transcription of AQP7. Although the pathways of AQP7 expression by insulin and its receptor signaling have not been elucidated yet, the similarity of the signal pathways between AQP3 and AQP7 expression controlled by insulin seems clear. Further studies are needed to elucidate the detailed mechanisms of the control of transcription of AQP3 mRNA, including identification of the signal transducers and specification of the promoter regions.

**REFERENCES**