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The effect of glucose, adenosine-5'-triphosphate, and adenosine on glucose transporter 9 expression in Madin-Darby Canine Kidney (MDCK) cells

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ABSTRACT

Objective: This study aimed to investigate the effect of glucose, adenosine triphosphate (ATP), and adenosine and their concentration dependence on glucose transporter 9 (GLUT9) expression in hyperglycemic conditions. These compounds have been reported to be associated with hyperglycemia in both animal and human. Extracellular ATP and adenosine exert their activities to regulate the pathophysiology through purinergic signaling. Their involvement was evaluated using non-selective antagonists such as caffeine (for P1 receptor) and suramin (for P2 receptor). Purinergic P1 receptors are associated with adenylate cyclase leading to increase or decrease the content of intracellular cAMP that plays a role in protein kinase A (PKA) activation. **Materials and Methods:** The GLUT9 mRNA and protein levels were measured by reverse transcription-polymerase chain reaction and Western blotting analysis, respectively. **Results:** The GLUT9 protein levels were increased significantly when the cells were treated with 25 mM and 50 mM glucose, in a concentration-dependent manner. The protein levels were also increased when cells were treated with 100 μM ATP and 100 μM adenosine for 6 h, whereas only adenosine decreased GLUT9 mRNA levels. Further study of adenosine-induced GLUT9 expression revealed that the observed effect was inhibited by caffeine. Moreover, stimulation and inhibition of PKA could decrease and increase the GLUT9 protein levels, respectively. **Conclusion:** Therefore, glucose could increase GLUT9 protein levels, and extracellular ATP and adenosine might be major contributing factors in enhancing the GLUT9 protein expression through purinergic system preferentially through P1 receptor activation and PKA inhibition.

Keywords: Glucose transporter 9, SLC2A9, expression, purinergic system

INTRODUCTION

A facilitative diffusion glucose transporter 9 (GLUT9) encoded by solute carrier family 2 member 9 gene (*SLC2A9*) plays a role in the transportation of glucose, fructose, and uric acid. In GLUT9-overexpressing *Xenopus* oocytes, it was identified to have a high affinity for glucose and fructose, and a high capacity for uric acid.[1-3] The transport activity depended on the electrical gradient when cell membrane potential and extracellular chloride ion were changed.[4] GLUT9 is strongly expressed in the kidney and plays a key role in glucose and uric acid homeostasis by re-absorption processes.[1,5] Filtered glucose and uric acid from glomeruli are reabsorbed into the blood circulation through many renal transporters.[6] Interestingly, GLUT9 is the only transporter that can carry both compounds and be expressed on apical as well as basolateral sides of renal tubular cells.[7] Thus, GLUT9 expression was hypothesized to involve a connection between hyperglycemia and hyperuricemia. Previous evidence had shown that pre-diabetes and insulin resistance in humans were related to an increase in serum uric acid.[8,9] Studies in *SLC2A9* transgenic mice found that renal clearance of uric acid was significantly decreased, whereas hyperuricosuria and early-onset nephropathy were found in *SLC2A9* knockout mice and mutation in humans and dogs.[3,5,10-12] From present observations, hyperglycemia seems to have an influence on GLUT9 protein levels. Using diabetic animal models, such as streptozotocin-induced diabetic rats and *ob/ob* mice, it was shown that GLUT9 protein levels were noticeably increased in the kidney, but the mRNA levels were not significantly changed in *ob/ob* mice.[13,14]

Besides the expression of GLUT9 found in the kidney, it could also be found in the placenta and be identified to contribute to embryo implantation.[1] Furthermore, the GLUT9 protein in human term placenta was raised in gestational diabetes.[15]

Hyperglycemia is a major cause of complications in diabetic patients. The American Diabetes Association guideline considers the subject with the fasting glucose concentration of more than 100 mg/dl to be hyperglycemic; whereas, one with glucose concentration above 126 mg/dl as diabetic.[16] Glucose is generated mainly as adenosine triphosphate (ATP), a precursor of cellular energy, which could be degraded to ADP, AMP, and adenosine. These compounds are also increased in hyperglycemic conditions. For example, adenosine was found to increase in glomeruli of diabetic rats to a six-fold higher level than in normal rats.[17] Moreover, ATP and adenosine are increased in cell culture models under long-term glucose treatment at high concentration. The ATP secreted from rat mesangial cells cultured in 25 mM glucose for 72 h was almost twice as high as that in 5.5 mM glucose, but total intracellular ATP levels were not altered.[18] Similarity, extracellular ATP was significantly increased in HK-2 cells cultured in high glucose conditions for 48 h.[19] The extracellular adenosine of rat podocytes cultured with 30 mM glucose was found to be significantly increased.[20] Furthermore, these compounds have been reported to influence cellular pathophysiological properties, such as pancreatic beta-cell proliferation, insulin secretion, insulin intolerance, and glucose uptake through purinergic receptors on cell membranes.[21-23] Based on these observations, the extracellular ATP and adenosine seem to be modulators of hyperglycemic conditions that might be involved in GLUT9 expression; however, it remains unclear. Therefore, this study intended to investigate the expression of GLUT9 mRNA and protein in response to hyperglycemic conditions, especially through the effect of ATP and adenosine. These observations may provide a better understanding of the relevant effect of hyperglycemia and related compounds on GLUT9 expression.

MATERIALS AND METHODS

Cell Culture

MDCK (NBL-2) cells (American Type Culture Collection, MD, USA), passage number in the range 65–82, were cultured in minimum essential medium (MEM) (MEM; Invitrogen Corporation, NY, USA) supplemented with 10% (v/v) fetal bovine serum (Invitrogen Corporation, NY, USA) and maintained in humidified atmosphere of 5% CO_2 at 37°C. The cells were seeded at a density of 20,000 cells per cm^2 and cultured for 4 days before GLUT9 mRNA, and protein levels were evaluated.

MTT Assay

The cells were treated with glucose (Merck, Darmstadt, Germany) and sorbitol (BDH, Poole, UK) at concentrations of 12.5, 25, and 50 mM for 72 h. The ATP (Calbiochem, MA, USA), adenosine (Sigma Chemical, MO, USA), and other compounds used to investigate the involvement of signaling pathways were treated for 24 h. After treatment, the treated medium was replaced with MTT solution at a concentration of 0.2 mg/ml. Intracellular MTT was reduced by mitochondrial reductase in viable cells to generate purple formazan crystals [24]. The crystals were further dissolved with dimethyl sulfoxide and absorbance measured at 570 nm.

Osmolality Determination

Either glucose or sorbitol was dissolved in the MEM containing 10% (v/v) fetal bovine serum. The osmolality, which was calculated with comparative measurements from the freezing points of pure water and of the samples, was measured by an automatic cryoscopic osmometer. The osmolality values were compared with the control group: MEM containing 5.5 mM glucose without sorbitol.

Treatments

The MDCK cells were treated with glucose and sorbitol at concentrations of 12.5, 25, and 50 mM for 72 h and the treatment was replaced every 24 h, before the GLUT9 protein was determined. The ATP and adenosine, at concentrations of 100 μM, were examined to evaluate the time response at 3, 6, 12, 18, and 24 h. Moreover, their concentration responses were also determined with a range of 25–400 μM for 6 h. The ATP was preferentially recognized with the purinergic P2 receptor; whereas, the adenosine was definitely bound to the purinergic P1 receptor. Their antagonists, suramin (for P2 receptor) and caffeine (for P1 receptor) were used to treat cells for 6 h with or without the substrates. The GLUT9 mRNA and protein levels were then determined. Moreover, the effect on GLUT9 protein levels of protein kinase A (PKA), a signaling protein of the P1 receptor, was evaluated using activators 10 μM 8-(4-chlorophenylthio) adenosine 3',5'-cyclic monophosphate (8-CPT) and 2 μM forskolin, as well as an inhibitor, 10 μM H-89. These compounds were incubated alone for 6 h or for 30 min before cotreatment with 100 μM adenosine for 6 h.

Reverse Transcription-polymerase Chain Reaction Analysis for mRNA Expression of *SLC2A9* Gene

Treatment with Trizol® reagent (Invitrogen Corporation, NY, USA) was performed to isolate RNA from MDCK cells. 1 μg of total RNA was reverse-transcribed and amplified by Omniscript® reverse transcription kit and *Taq* DNA polymerase, respectively, following the manufacturer's instructions (Qiagen, Hilden, Germany). Primers were designed from the NCBI primer design tool to recognize the *SLC2A9* under NCBI reference sequence of NM_001130835.2. The forward primer was 5'-TGA GAA GCA TGA CCA GGC AG-3' and the reverse primer was 5'-TCA GCC CAA AGC CAC CTA TG-3'. Glyceraldehyde 3-phosphate dehydrogenase was used as a housekeeping gene for normalization. The cycling protocol of cDNA amplification was designed for 35 cycles and performed using Mastercycler 5333 (Eppendorf, Hamburg, Germany). It consisted of denaturing at 95°C for 45 s, annealing at 55°C for 45 s, and extending the temperature to 72°C for 45 s, then final extending the temperature to 72°C for 10 min. The amplified products were fractionated at a constant 80 V for 1 h on 2% (w/v) agarose gel before being stained by ethidium bromide. The stained products were determined by gel documentation, and the intensity of each band was analyzed by Image J software.

Western Blotting Analysis for GLUT9 Protein Expression

The cells were lysed by RIPA buffer containing 50 mM Tris-hydrochloride (pH 8.0), 150 mM sodium chloride, 0.1% (w/v) sodium dodecyl sulfate (SDS), 0.5% (w/v) sodium deoxycholate, 1% (v/v) Triton X-100, and protease inhibitor cocktail. The cell lysates were centrifuged at 12,000 rpm, 4°C for 15 min, and then 10 μg of total proteins in the supernatant were loaded on 12.5% (w/v) SDS polyacrylamide gel. The protein fractions were separated with a constant 80 V for 3 h. The separated proteins were transferred onto a PVDF membrane with a constant 60 V for 2 h. The membrane was blocked by 5% (w/v) bovine serum albumin in phosphate buffer saline with 0.1% (v/v) Tween20 and then was incubated overnight with rabbit polyclonal anti-GLUT9 antibody ab82910 (1:3,000) (Abcam, Cambridge, UK) at 4 °C. Horseradish peroxidase-coupled goat polyclonal secondary antibody against rabbit IgG ab6721 (1:5,000) (Abcam, Cambridge, UK) and horseradish peroxidase-coupled goat monoclonal anti-actin antibody ab20272 (1:10,000) (Abcam, Cambridge, UK) were incubated for 2 h to determine GLUT9 and actin proteins, respectively. The protein detection on the membrane was performed using Luminata™ Crescendo kit (Millipore Corporation, MA, USA) and detected by gel documentation. The intensity of each band was analyzed by Image J software.

Statistical Analysis

Statistical analysis was performed using the GraphPad Prism software version 5. All values were expressed as mean ± SEM, which were calculated from at least three independent experiments. Each experiment was conducted in duplicate. Statistical differences were analyzed by one sample *t* test when each treatment was compared with the value of control group =1. On the other hand, one-way analysis of variance and Dunnett's multiple comparison post-test was used to compare GLUT9 expression between 100 μM adenosine-alone treatment and cotreatment with activators or inhibitors. In all cases, $P < 0.05$ was considered to be statistically significant.

RESULTS

Cell Viability

This experiment was performed to determine the concentrations of examined compounds that caused no change in viability of MDCK cells. These non-toxic concentrations were used in further studies. Hence, the observed effects were the results of the experimental conditions chosen to not alter cell viability. The percentage of viable cells was >90% and was not significantly different from control.

Osmolality Determination

The osmolality value of MEM medium was equal to 306.33 ± 2.71 mOsmol/Kg, which was similar to the physiological osmolality of plasma. The glucose concentrations of 12.5, 25, and 50 mM increased the osmolality values significantly to 314.83 ± 2.02, 326.50 ± 1.69, and 348.75 ± 1.66 mOsmol/Kg, respectively. Similarly, sorbitol, a slow-metabolizing sugar, at concentrations of 12.5, 25, and 50 mM, increased the osmolality values significantly to 321.78 ± 3.82, 327.45 ± 3.29, and 356.22 ± 3.95 mOsmol/Kg, respectively. There was no statistically significant between the increased osmolality values caused by glucose and that of sorbitol at the same concentration.

Effect of Glucose and Hyperosmotic Stress on GLUT9 Protein Levels

Figure 1 illustrates that GLUT9 protein levels were enhanced in cells treated with glucose in a concentration-dependent manner. The proteins were increased significantly by 1.36 ± 0.12-fold and 1.78 ± 0.15-fold when the cells were treated with 25 mM and 50 mM glucose for 72 h, respectively. In contrast, cells treated with 12.5, 25, and 50 mM sorbitol showed no significant change of GLUT9 protein levels [Figure 2].

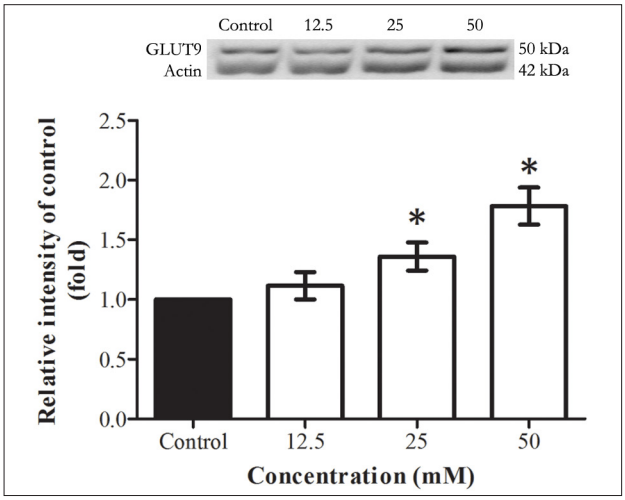

Figure 1: Concentration-response of glucose-induced increase of GLUT9 protein levels when cells were treated for 72 h. The upper photograph showed the results of one representative experiment. Each value is represented by the mean ± SEM (*, *p*-value < 0.05, n = 5–6).

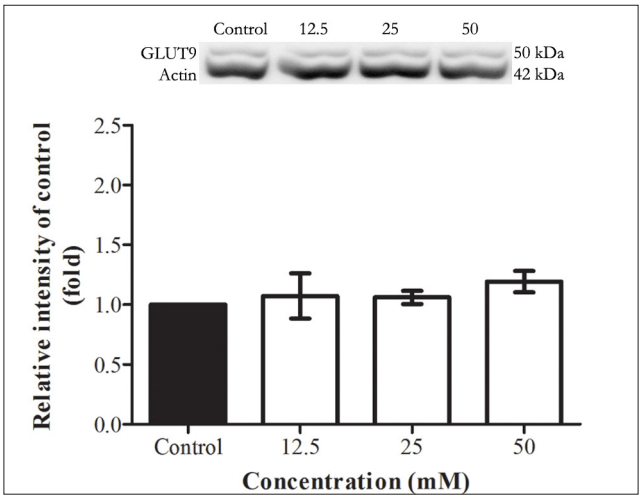

Figure 2: Concentration-response of sorbitol on the GLUT9 protein level when cells were treated for 72 h. The upper photograph shows the results of one representative experiment. Each value is represented by the mean ± SEM (n = 3–4).

Effect of ATP and Adenosine on GLUT9 Expression

Solutions of ATP and adenosine (100 μM) were applied to determine the time-response of GLUT9 mRNA and protein levels. As seen in Figure 3a, the GLUT9 mRNA levels were unchanged by ATP during 24-h periods. However, the GLUT9 protein levels were increased significantly after treatment for 3, 6, and 12 h, reaching the maximum level at 6 h. After 18 h, the GLUT9 protein was decreased to the control level. Figure 3b illustrates the response of GLUT9 mRNA and protein levels to adenosine. The GLUT9 mRNA levels were decreased significantly after treatment with adenosine for 6 h, and then this effect was recovered to the control level after 12 h. Although the mRNA levels were decreased, the GLUT9 protein levels were increased significantly when cells were treated with adenosine for 6 h. On the other hand, when MDCK cells were treated for 18 h, the GLUT9 protein levels were decreased significantly. These results revealed that alterations of the GLUT9 expression due to adenosine were discrepant between levels of mRNA and protein, particularly after 6 h.

Based on our results of the 6-hour treatment conditions, concentration-response of ATP and adenosine were determined. The level of GLUT9 proteins was significantly increased by treating with ATP at concentrations of 50 μM and 100 μM, whereas, this effect was decreased to control level at ATP concentrations of 200 μM and 400 μM. On the other hand, adenosine only at the concentration of 100 μM could statistically significant increased the protein level ($P < 0.05$). Moreover, we further evaluated the involvement of purinergic receptors in ATP- or adenosine-induced GLUT9 protein expression. An antagonist of the P2 receptor, suramin, was used to explore the effect of ATP on the GLUT9 protein level. It was found that 100 μM suramin had no effect on the GLUT9 protein levels when compared with either the control group or the ATP-treated group (data not shown). To see the effect of adenosine on GLUT9 expression, alterations of the mRNA and protein expressions were determined using caffeine, an

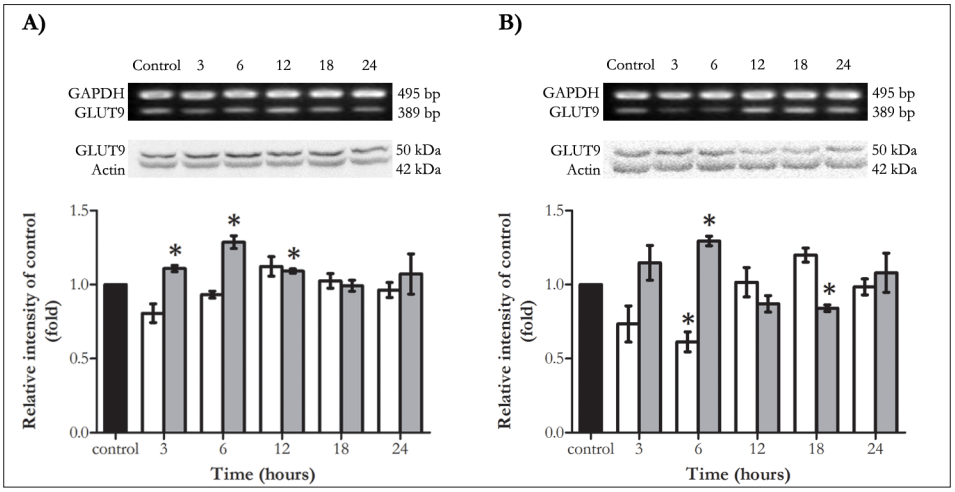

Figure 3: Effect of exogenous ATP (A) and adenosine (B) at concentrations of 100 μM on the levels of GLUT9 mRNA (open bar) and protein (closed bar). The upper photograph shows the results of one representative experiment for ATP and adenosine. Each value is represented by the mean ± SEM (*, p-value < 0.05, n = 4–6).

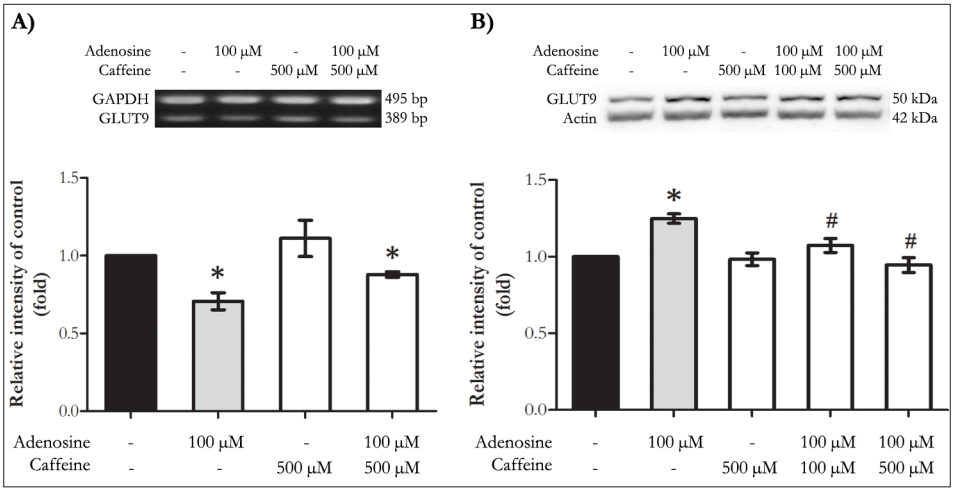

Figure 4: Effect of caffeine on the levels of GLUT9 mRNA (A) and protein (B). The upper photograph shows the results of one representative experiment. Each value is represented by the mean ± SEM (*, p-value < 0.05 vs. control group; #, p-value < 0.05 versus 100 μM adenosine-alone treatment, n = 3–6).

antagonist of the purinergic P1 receptor. The GLUT9 mRNA and protein levels were not altered by 500 μM caffeine treatment alone (Figure. 4). Furthermore, treated combination between adenosine and caffeine unchanged the level of GLUT9 mRNA when compared with the adenosine-treated group [Figure 4a]. However, Figure 4B illustrates that increased GLUT9 protein levels due to adenosine were significantly inhibited by caffeine at concentrations of 100 μM and 500 μM ($P < 0.05$).

PKA Involved with the Expression of GLUT9 Protein

PKA, a key signaling protein of the P1 receptor, was examined and the results presented in Figure 5. When MDCK cells were treated with 10 μM 8-CPT, a cAMP analog, with and without 100 μM adenosine, the GLUT9 protein levels were decreased significantly compared with adenosine treatment alone and the control group, respectively ($P < 0.05$). Another PKA activator, 2 μM forskolin, could reduce the GLUT9 protein levels in both with- and without-adenosine conditions but it was not statistically significant. On the other hand, the GLUT9 protein levels were increased slightly by treated with 10 μM H-89, a PKA inhibitor. Moreover, the combination of adenosine and H-89 could significantly increase the GLUT9 protein levels when compared with control and adenosine treatment alone.

DISCUSSION

GLUT9 transporters play roles in glucose uptake into the renal tubular cells as well as efflux from the cells into the bloodstream. Furthermore, not only glucose is allowed to be transported through GLUT9, but uric acid is also re-absorbed.[2] This suggests that it might be a key connector between hyperglycemic and hyperuricemic development. For understanding possible control mechanism of GLUT9 expression, this study covered *in vitro* assays of the effect of the hyperglycemic condition, that is, increased glucose concentration, osmolality, ATP and adenosine, on GLUT9 expression in MDCK cells, derived from the kidney of cocker spaniel, a renal tubular cell model. In our result, the GLUT9 protein levels were dramatically increased by glucose in a concentration-dependent manner. This finding confirmed the idea that the GLUT9 proteins are raised in hyperglycemia, which involves the increase of glucose level.[13-15] Other glucose transporters, SGLT2 and GLUT2, have been also reported to increase in hyperglycemic condition. Present medication such as SGLT2 inhibitors could decrease the fasting plasma glucose level in diabetic patients, and some of them are effective in moderate renal impairment.[25] However, SGLT2 and GLUT2 proteins expressed specifically on the apical and basolateral sides of renal tubular cells, respectively, whereas the GLUT9 proteins were exhibited on both sides.[7] These observations suggest that inhibition of glucose transporters especially GLUT9 in the kidney has a potential role for further development as a treatment for diabetes mellitus and reduced risk of hyperuricemia. Moreover, the concentrations of glucose had an influence on osmolality similar to sorbitol mimicking a hyperosmotic stress effect, but the increased GLUT9 protein levels were not due to alterations of osmolality values [Figure 2]. Therefore, these results suggested that the high amount of glucose, but not hyperosmotic stress, might contribute to increased GLUT9 protein levels.

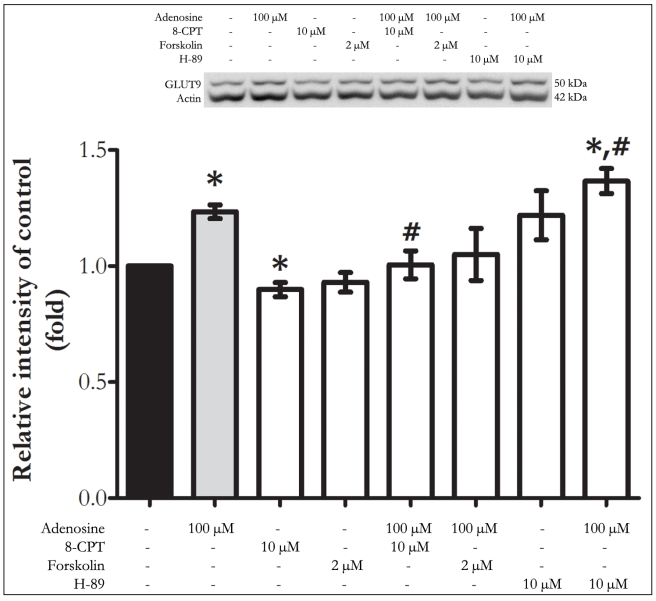

Figure 5: Effect of protein kinase A activator and inhibitor on the levels of GLUT9 protein. The upper photograph shows the results of one representative experiment. Each value is represented by the mean ± SEM (*, p-value < 0.05 versus control group; #, p-value < 0.05 versus 100 μM adenosine-alone treatment, n = 5–7).

Although GLUT9 protein levels may be enhanced directly by high concentrations of glucose, the products of glucose metabolism, particularly ATP, may also be involved. Intracellular ATP could be released when there was a change in physiological conditions, such as high glucose treatment. Extracellular ATP has been reported to exert potent autocrine and paracrine effects on cellular function through the purinergic system.[22] The purinergic receptors consist of two major classes, P1 and P2, based on their specific ligands, adenosine and ATP, respectively. Recently, the purinergic system has been shown to be involved in diabetes, and these receptors' expressions were identified, as well as being found in kidney cells, including MDCK cells.[26,27] Our investigations indicated that the GLUT9 protein levels were increased to reach a maximum level after 6 h when MDCK cells were treated with 100 μM ATP. This response period was concordant with previous observations which demonstrated that the maximum concentration of ATP affecting on glucose uptake was 100 μM.[28,29] Primary rabbit renal proximal tubular cells treated with ATP at the concentrations of 1 and 100 μM for 6 h increased significantly glucose uptake but at the concentration of 1000 μM exhibited lower response than that of 100 μM.[28] A study in myoblast C_2C_{12} cells, which determined glucose uptake caused by 0.1–1000 μM ATP under added cytochalasin B condition, found that glucose uptake was increased significantly and reached maximum response when the cells were treated with ATP at the concentration of 100 μM.[29] Although cytochalasin B was generally known to inhibit GLUTs activities, Augustin *et al*. demonstrated that GLUT9 activity was not inhibited.[1] Enhancement of GLUT9 protein levels caused by ATP might be related to the increasing efficiency of glucose reabsorption in the kidney. However, GLUT9 mRNA levels were not altered by adding ATP to the cells. Previous studies in bEnd.3 cells also

demonstrated that neither increased ATP nor ATP depletion had an effect on GLUT9 mRNA levels.[30] Therefore, our further study was focused on the detailed mechanism of ATP-induced GLUT9 expression at the protein level. Our results revealed that alteration of GLUT9 protein levels was unlikely to be involved in the P2 receptor, since there was no inhibitory effect of suramin, a P2 antagonist, and on ATP-induced GLUT9 protein expression. These findings could be explained by the fact that ATP was immediately degraded by brush-border enzymes on MDCK cells; therefore, it could not exert its activity by itself.[23,27]

The content of adenosine as a metabolite of ATP was increased 6-fold in the glomeruli of streptozotocin-induced diabetic rats.[17] Extracellular adenosine has been reported that it regulates cellular function and contributes to the pathophysiology of diabetes through the P1 receptor.[23] Thus, one of the possible factors that might cause the increasing of GLUT9 protein levels after treatment with ATP was adenosine. The present study demonstrated initially that 100 μM adenosine increased the GLUT9 protein levels through the P1 receptor after treatment for 6 h, since they were inhibited by caffeine, a P1 antagonist. Our finding correlated with the study of Patinha *et al*., in 2014, which reported that renal glucose excretion in diabetic rats was decreased after administration of an adenosine analog.[31] In contrast, the GLUT9 mRNA level was significantly decreased by 100 μM adenosine after treatment for 6 h. This result might involve in reduced protein level after treatment for 18 h. The absence of correlation between the GLUT9 levels in term of mRNA and protein after treatment with adenosine may occur due to post-transcriptional and post-translational modifications without affecting gene expression. Non-coding RNAs such as miR-153 could be a possible factor that involved in this finding. The miR-153 which exhibited in *Canis lupus familiaris* played a role in mRNA stability and translational regulation. Bahn *et al*. demonstrated that miR-153 played an important role in the regulation of GLUT9 expression [32]. The miR-153 overexpressing pancreatic beta cells such as MIN6 (mouse) and 1.1B4 (human) were used to evaluate GLUT9 expression using luc-GLUT9-UTR as well as luciferase assay. The expression of miR-153 was decreased significantly when both cells were cultured in high glucose condition. However, GLUT9 protein was significantly increased. In addition, the GLUT9 protein expression in HepG2 cells that transfected with 153-anti-miRNAs was also significantly increased.[33] These observations suggested that miR-153 might involve in regulation of GLUT9 expression in hyperglycemic condition. Therefore, adenosine which expected to be a contributing factor of hyperglycemic condition might regulate GLUT9 translation through miR-153. However, transcriptional regulation of GLUT9 might not be influenced by miR-153; therefore, the level of GLUT9 mRNA was decreased in our experiment. This point still needs to be investigated for a better understanding. The mechanism underlying the decreasing of GLUT9 mRNA levels should be studied further.

Purinergic P1 receptors coupled with G-protein were divided into A_1, A_{2a}, A_{2b}, and A_3 subtypes. The adenosine receptors A_1 and A_3 inhibited the activity of adenylate cyclase through $G_{\alpha i}$ protein, leading to decrease of intracellular cAMP content, whereas A_{2a} and A_{2b} subtypes stimulated this enzyme through $G_{\alpha s}$ protein.[34] cAMP played a role in triggering PKA, which is a key signaling protein for the P1 receptor. Therefore, the involvement of PKA in GLUT9 protein expression through the P1 receptor was investigated in this study. Our results indicated that the GLUT9 protein levels were down-regulated by 8-CPT, a cAMP analog, and the increase of GLUT9 protein level due to adenosine was inhibited. Forskolin, an alternative PKA activator, which could be stimulated indirectly through adenylate cyclase activation,[35] was used. It seemed to decrease the GLUT9 protein levels, but the effect was not significant [Figure 5]. These results suggest that PKA stimulation could inhibit the effect of adenosine on GLUT9 protein expression in MDCK cells. On the other hand, the increase of GLUT9 protein level caused by adenosine was further enhanced by the presence of H-89, a PKA inhibitor. Therefore, PKA inhibition might be related to the effect of adenosine on increasing GLUT9 protein levels. These results suggest that adenosine could increase GLUT9 protein levels through A_1 and/or A_3 subtypes of the P1 receptor. A previous report has demonstrated that the stimulation of A_1 subtypes in muscle cells of diabetic rats could induce glucose uptake in insulin-independent conditions.[36] In spite of the fact that GLUT9 protein was slightly expressed in muscle cells, this observation seems to explain the possible relevance of the P1 receptor, particularly the A_1 subtype, to glucose uptake in peripheral tissues.[13,36]

CONCLUSION

The expression of GLUT9 in term of protein level was increased significantly by glucose, ATP, and adenosine in MDCK cells. The purinergic P1 receptor, which preferentially recognized adenosine, was involved. Moreover, the PKA inhibition increased further the GLUT9 protein levels caused by adenosine, but the PKA stimulation decreased this effect. Therefore, extracellular adenosine could be a major contributing factor in enhancing GLUT9 protein expression through the $G_{\alpha i}$ protein-coupled P1 receptor in hyperglycemic conditions.

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CONFLICTS OF INTEREST

Jamras Kanchanapiboon declares that he has no conflicts of interest. Thitima Pengsuparp declares that she has no conflicts of interest.

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