Effects of Angiotensin-Converting Enzyme Inhibitors on Glucose Uptake

Akira Kudoh, Akitomo Matsuki

Abstract—We investigated the effect of angiotensin-converting enzyme inhibitors on glucose uptake regulation as well as the effect of bradykinin (BK) on glucose uptake and its regulation by using inhibitors of phospholipase C, BK B2 receptor, protein kinase C, phosphatidylinositol 3-kinase, tyrosine kinase, and intracellular Ca²⁺. We measured 2-deoxyglucose uptake by using L₆ skeletal muscle cells. In the presence of 1 nmol/L of insulin, 1 μmol/L of enalaprilat enhanced insulin-induced glucose uptake from 89.2±8.1 to 138.0±13.6 pmol/h per mg protein. The stimulation of glucose uptake with enalaprilat was blocked to 92.7±7.8 pmol/h per mg protein by 10 μmol/L HOE 140 (a BK B2 receptor antagonist). In the presence of 1 nmol/L of insulin, exposure to 10 μmol/L BK stimulated glucose uptake from 89.2±8.1 to 171.6±10.1 pmol/h per mg protein. However, in the absence of insulin, BK could not enhance glucose uptake. One hundred nanomoles per liter of tyrphostin A-23 and genistein, which are tyrosine kinase inhibitors, significantly decreased the BK-induced glucose uptake from 142.0±8.4 to 87.6±6.4 and 85.2±7.3 pmol/h per mg protein, respectively. BK-induced glucose uptake was inhibited significantly by 10 μmol/L U73122 (a phospholipase C antagonist) from 142.0±8.4 to 95.7±9.5 pmol/h per mg protein. One and 20 μmol/L of TMB-8 (an intracellular calcium antagonist) significantly decreased BK-induced glucose uptake from 142.0±8.4 to 108.0±9.6 and 100.8±11.4 pmol/h per mg protein. Angiotensin-converting enzyme inhibitors enhanced insulin-induced glucose uptake via the BK B2 receptor. BK-stimulated glucose uptake is related to phospholipase C, tyrosine kinase, and an increase in intracellular calcium. (Hypertension. 2000;36:239-244.)

Key Words: angiotensin-converting enzyme inhibitors ■ bradykinin ■ glucose ■ calcium ■ phospholipases ■ protein kinases

A close association between hypertension and insulin resistance has been suggested. Patients with essential hypertension are resistant to insulin-stimulated glucose uptake.¹ Altered glucose metabolism may be related to hemodynamic factors, including circulating vasoactive agents.² Forty percent of individuals with non-insulin-dependent diabetes mellitus are hypertensive and have an increased risk of cardiovascular disease.³ Therefore, there is considerable interest in antihypertensive drugs that improve not only high blood pressure but also glucose tolerance and insulin resistance. Angiotensin-converting enzyme (ACE) inhibitors improve sensitivity to insulin.⁴ After therapy with captopril in hypertensive patients, insulin-mediated glucose disposal is increased by 15% to 20% of baseline.⁵ In 130 patients with non-insulin-dependent diabetes mellitus and hypertension, captopril treatment led to a decrease in plasma glucose.⁶ This action of ACE inhibitors is mediated in part by bradykinin (BK), because BK enhances glucose uptake.⁷ Some authors have demonstrated the mechanisms of BK-induced glucose uptake.⁸–⁹ BK may be involved in contraction-stimulated and/or exercise-stimulated glucose transport.⁹ However, the mechanisms of BK on glucose transport remain unclear.

BK acts on G-protein–coupled receptors and activates phospholipase C (PLC), which stimulates the rapid formation of inositol-1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG).¹⁰ PLC has been found to mimic insulin action to increase glucose transport in skeletal muscle.¹¹ IP₃ increases the intracellular Ca²⁺ concentration. An increase in intracellular Ca²⁺ concentration is also involved in the enhancement of glucose transport.¹² DAG acts as the second messenger to produce protein kinase C (PKC). Activation of DAG and PKC stimulates glucose transport.¹³ The major stimulation for glucose uptake is insulin, which binds to the insulin receptors of the cell surface and induces the autophosphorylation of its β subunit, which in turn phosphorylates insulin receptor substrate-1 on tyrosine residues and leads to activation of phosphatidylinositol 3-kinase. Activation of phosphatidylinositol 3-kinase leads to the translocation of glucose transporter type 4, which transports glucose across membranes.¹⁴ The PLC and phosphatidylinositol 3-kinase share inositol 4,5-bisphosphate as their substrate;¹⁵ this may be closely related between the 2 systems. We investigated the effect of ACE inhibitors on glucose uptake as well as the effect of BK on glucose uptake and its regulation with inhibitors of PLC, BK

Received January 11, 2000; first decision January 31, 2000; revision accepted February 24, 2000.
From the Department of Anesthesiology, University of Hirosaki School of Medicine, Aomori, Japan.
Correspondence to Akira Kudoh, MD, 5 Zaifucho, Hirosaki 036, Aomori, Japan.
© 2000 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org
B2 receptor, PKC, phosphatidylinositol 3-kinase, tyrosine kinase, and intracellular Ca2+. Because skeletal muscles are the major target tissues for glucose use, we used L6 skeletal muscle cells. From these results, we concluded that ACE inhibitors enhance insulin-induced glucose uptake via the BK B2 receptor and that BK-stimulated glucose uptake is associated with activation of tyrosine kinase and PLC and an increase in intracellular Ca2+.

**Methods**

**Cell Culture**  
L6 muscle cells were grown in α-minimum essential medium containing 2% fetal bovine serum and antibiotic antimycotic solution (100 U/mL penicillin G, 100 μg/mL streptomycin, 250 ng/mL amphotericin B) at 37°C in an atmosphere of 5% CO2 and 95% air. The cells were grown in 12-well plates. The cells were incubated in DMEM 24 hours before glucose uptake was measured and for the last 5 hours with 5.6 mmol/L glucose.

**2-Deoxyglucose Uptake**  
Two hours before the experiment, 10 mmol/L 2-deoxyglucose (2-DG) was added to the glucose culture medium so that basal uptake of [3H] 2-DG was lowered during the subsequent assay. Cells were treated with U 73122 (a PLC antagonist), genistein and tyrophostin A-23 (tyrosine kinase inhibitors), tyrophostin A1 (the inactive analogue of tyrosine kinase), TMB-8 (an intracellular calcium antagonist), nifedipine (a Ca2+ channel blocker), wortmannin (a phosphatidylinositol 3-kinase antagonist), and staurosporine (a PKC antagonist) for 30 minutes after exposure to 1 nmol/L insulin for 30 minutes. One hundred nanomoles per liter of BK was then added, and the cells were incubated for 30 minutes.

2-Deoxyglucose uptake was assayed in medium containing 6.0 mmol/L KCl, 0.2 mmol/L Na2HPO4, 1.4 mmol/L MgSO4, 1.0 mmol/L CaCl2, 1.0 mmol/L NaHPO4, and 10.0 mmol/L HEPES-NaOH to yield a pH of 7.4 and 2% bovine serum albumin. The tracer (1 μCi/mL [3H]2-DG) was added for 5 minutes together in addition to 0.2 μCi/mL [3H] glucose. During the incubation, the transport was stopped by the addition of 400 mL/well of ice-cold 600 mL/M glucose. The dishes were transferred to ice, and the monolayers were washed twice with 3 mL/well of ice-cold 200 mL/M glucose. 2-DG uptake was counted in a liquid scintillation counter 60 minutes after the addition of 1 mL/well of 0.2 mol/L NaOH. Protein was determined by the method of Bradford with bovine serum albumin as standard.

**Solutions and Drugs**  
BK, insulin, TMB-8, wortmannin, and staurosporine were purchased from Sigma Chemical Co. U 73122 was purchased from Biomol Research Laboratories. Genistein, tyrophostin A-23, and tyrophostin A1 were purchased from Calbiochem-Novabiochem Co. 2-deoxy-D-[3H]glucose was purchased from Amersham. Genistein, tyrophostin A-23 and tyrophostin A1, U 73122, staurosporine, and wortmannin were dissolved in 0.1% dimethyl sulfoxide.

**Statistical Analysis**  
Results are expressed as mean±SEM. Each experiment was performed in triplicate. Every experiment was repeated 8 times with different cell preparations. The significance of differences was determined with ANOVA. When a significant F value was obtained, comparisons of means were done with the Student t test for paired and unpaired samples, the Bonferroni test, and the Student-Newman-Keuls multiple comparison test. Differences in mean values were considered significant at P<0.05.

**Results**  
Insulin stimulated 2-deoxyglucose uptake in L6 skeletal muscle cells in a dose-dependent manner. 2-Deoxyglucose uptake increased from a basal level of 26.6±2.7 to 209.4±14.5 pmol/h per mg protein in the presence of 50 nmol/L insulin for 30 minutes.

We examined the effects of enalaprilat on insulin-induced glucose uptake. Cells were incubated in 1 μmol/L enalaprilat for 30 minutes and then were incubated with concentrations of 1 or 10 nmol/L of insulin for 30 minutes. The stimulation of glucose uptake with enalaprilat was blocked to 92.7±7.8 pmol/h per mg protein by 10 μmol/L of HOE 140. In the presence of 10 nmol/L of insulin, enalaprilat enhanced insulin-induced glucose uptake from 89.2±8.1 to 138.0±13.6 pmol/h per mg protein. The stimulation of glucose uptake with enalaprilat was blocked by HOE 140 (Figure 1).

Figure 2 shows the effect of BK on glucose uptake in the presence of 1 nmol/L of insulin. When the cell was exposed to BK for 30 minutes, the BK-stimulated glucose uptake was at its maximum. Thus we examined the effects of BK on glucose uptake after exposure to BK for 30 minutes.

In the presence of 1 nmol/L of insulin, exposure to BK stimulated insulin-induced glucose uptake from 89.2±8.1 in the absence of BK to 171.6±10.1 pmol/h per mg protein in 10 μmol/L of BK in a dose-dependent manner. In the presence of 10 nmol/L of insulin, BK stimulated insulin-induced glucose uptake in a dose-dependent manner. How-
ever, in the absence of insulin, 10 μmol/L of BK did not enhance glucose uptake (Figure 3).

We examined the effects of tyrosine kinase on BK-induced glucose uptake. One hundred nanomoles per liter of tyrphostin A-23 and genistein, which are tyrosine kinase inhibitors, significantly decreased the BK-induced glucose uptake from 142.0±8.4 to 87.6±6.4 and 85.2±7.3 pmol/h per mg protein, respectively. Tyrphostin A1, the inactive analogue, had no significant effect on basal or BK-stimulated IP₃ formation (Figure 4).

We examined the effects of PLC on BK-induced glucose uptake. When the cultures were exposed to 10 μmol/L of U73122, which is a PLC antagonist, BK-induced glucose uptake was significantly inhibited from 142.0±8.4 to 95.7±9.5 pmol/h per mg protein. Ten micromoles per liter of U73122 alone did not significantly decrease insulin-induced glucose uptake (Figure 5).

We examined the effects of intracellular calcium on BK-induced glucose uptake. The exposure to TMB-8 (an intracellular calcium antagonist) alone and nifedipine (a voltage-dependent Ca²⁺ channel blocker) alone did not affect insulin-induced glucose uptake. One and 20 μmol/L of TMB-8 significantly decreased BK-induced glucose uptake from 142.0±8.4 to 108.0±9.6 and 100.8±11.4 pmol/h per mg protein. One and 10 μmol/L of nifedipine did not cause a significant decrease in BK-induced glucose uptake (Figure 6).

We examined the effects of phosphatidylinositol 3-kinase and PKC on BK-induced glucose uptake. Wortmannin (a phosphatidylinositol 3-kinase antagonist) could significantly block insulin-induced glucose uptake but could not significantly block BK-induced glucose uptake. Staurosporine did not block BK-induced glucose uptake.
Discussion

This study showed that enalaprilat enhanced insulin-induced glucose uptake and that the stimulation was blocked by BK B2 receptor antagonist. This result indicates that enalaprilat-stimulated glucose uptake acts via the BK B2 receptor in skeletal muscle. ACE inhibitors are reported to improve insulin sensitivity through the action of BK. Dietz demonstrated that the effect of BK on glucose uptake may occur in skeletal muscle, because BK potentiates insulin-induced glucose uptake without affecting blood flow. Some authors also reported the involvement of BK on glucose uptake in several cell types. Therefore we studied the effect of BK on glucose uptake. This study showed that BK increased glucose uptake in the presence of insulin but not in the absence of insulin. Miyata et al suggested that glucose transporter type 4 translocations are not affected by BK alone, but that they are increased by BK in the presence of insulin. These findings suggest that BK appears to stimulate any step in the insulin signaling cascade.

BK signaling involves many pathways. BK receptors are coupled to PLC, which can increase the intracellular Ca$^{2+}$ concentration. PLC and phosphatidylinositol 3-kinase share inositol 4,5-biphosphate as their substrate; this may be closely related between the 2 systems. Interactions between protein tyrosine kinase and BK or PLC are also suggested. BK stimulates tyrosine kinase receptors, and the phosphorylation of PLC is blocked by tyrosine kinase inhibitors. Because PLC, tyrosine kinase, phosphatidylinositol 3-kinase, PKC, and intracellular calcium are known to stimulate glucose uptake, we studied the effect of these factors on BK-induced glucose uptake.

PLC has been found to mimic insulin action. BK activates PLC, which stimulates the rapid formation of IP$_3$, which in turn leads to an increase in intracellular Ca$^{2+}$. Elevation of the intracellular Ca$^{2+}$ concentration leads to increased glucose transport to skeletal muscle. We examined whether PLC is involved in BK-stimulated glucose transport. Our data showed that PLC inhibitors could not block insulin-induced glucose uptake but that it could inhibit BK-stimulated glucose uptake. This result indicates that BK-stimulated glucose uptake is partly associated with activation of PLC. IP$_3$ is unlikely to be associated with BK-stimulated glucose uptake. Lithium, which inhibits the degeneration of inositol phosphates, increases glucose transport. However, the stimulatory effects of lithium on glucose transport may not involve phosphatidylinositol metabolism.

![Figure 4: Effects of tyrosine kinase on BK-induced glucose uptake in L6 skeletal muscle cells.](image)

![Figure 5: Effects of PLC on BK-induced glucose uptake in L6 skeletal muscle cells.](image)
BK-stimulated glucose uptake reached a maximum at 30 minutes after stimulation. Because BK-induced IP$_3$ production reaches a maximum 20 to 30 seconds after stimulation, BK-stimulated glucose uptake is not dependent on IP$_3$. Thus BK-stimulated glucose transport appears to involve PLC but to act via a different pathway from that of the PLC-IP$_3$ sequence.

To test the hypothesis that intracellular Ca$^{2+}$ is involved in BK-stimulated glucose uptake, we examined the effect of TMB-8, an intracellular Ca$^{2+}$ antagonist, on BK-stimulated glucose uptake. TMB-8 blocks Ca$^{2+}$ channels and interferes with Ca$^{2+}$ release from the sarcoplasmic reticulum. Our findings indicated that TMB-8 inhibited BK-stimulated glucose uptake. On the other hand, nifedipine, a voltage-sensitive Ca$^{2+}$ channel blocker, had little effect on BK-stimulated glucose uptake. This evidence suggests that BK-stimulated glucose uptake is independent of any increase in Ca$^{2+}$ via voltage-sensitive Ca$^{2+}$ channels and is dependent on the release of Ca$^{2+}$ from the sarcoplasmic reticulum. Thus BK-stimulated glucose transport is partly associated with an alteration of intracellular Ca$^{2+}$.

Our data showed that inhibitors of tyrosine kinase inhibited BK-stimulated glucose uptake at low concentrations. Modifications of insulin-stimulated glucose transport are associated with parallel alterations in tyrosine kinase. Russ et al. suggested that insulin receptors are coupled to a GTP-binding protein, which acts as a regulator of tyrosine kinase activity.

BK stimulates tyrosine kinase activity via GTP-binding protein. Thus, the BK-tyrosine kinase cascade appears to play a significant role in BK-stimulated glucose uptake. In addition, activation of tyrosine kinase requires an elevation of intracellular Ca$^{2+}$. In this study, the fact that an intracellular Ca$^{2+}$ blocker inhibited BK-stimulated glucose uptake appears to support the theory that increased Ca$^{2+}$ levels are necessary for activation of tyrosine kinase.

Wortmannin is a potent and selective inhibitor of phosphatidylinositol 3-kinase and inhibits insulin-stimulated glucose transport. In this study, wortmannin could not block BK-induced glucose uptake, although the same concentration of wortmannin significantly blocked insulin-induced glucose uptake. BK-induced glucose uptake seems to act via a different pathway from that of phosphatidylinositol 3-kinase. Activation of PKC has been involved in the mediation of some of the action of insulin. Activation of PKC by phorbol esters is associated with increases in glucose transport in skeletal muscle, and downregulation of PKC inhibits stimulation of glucose uptake by insulin. However, we could not show a direct involvement of PKC in BK-induced glucose uptake in skeletal muscle. PKC regulates PLC activity: PKC inhibition stimulates PLC activity, and PKC activation inhibits PLC via a negative feedback mechanism. Thus, PKC inhibition with staurosporine might have activated PLC in this study.

This study was performed on cell cultures. The actions of BK on improving insulin sensitivity by ACE inhibitors may be mitigated in either animal or human models. Further studies are needed to provide a more complete evaluation of the mechanisms of enalaprilat-induced or BK-induced glucose uptake.

In conclusion, ACE inhibitors enhanced insulin-induced glucose uptake. The stimulation was blocked by the BK B2 antagonist. BK stimulated glucose uptake in the presence of insulin. Tyrosine kinase plays an important role in BK-stimulated glucose uptake, which is related to PLC and to an alteration of intracellular calcium concentration. However, phosphatidylinositol 3-kinase and PKC are not involved in the stimulation.

References


Effects of Angiotensin-Converting Enzyme Inhibitors on Glucose Uptake
Akira Kudoh and Akitomo Matsuki

Hypertension. 2000;36:239-244
doi: 10.1161/01.HYP.36.2.239

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/36/2/239

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/