Activation of p38 MAP kinase enhances sensitivity of muscle glucose transport to insulin

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Submitted 5 October 2004; accepted in final form 1 December 2004

Geiger, Paige C., David C. Wright, Dong-Ho Han, and John O. Holloszy. Activation of p38 MAP kinase enhances sensitivity of muscle glucose transport to insulin. Am J Physiol Endocrinol Metab 288: E782–E788, 2005. First published December 7, 2004; doi:10.1152/ajpendo.00477.2004.—Muscle contractile activity is followed by an increase in the sensitivity of glucose transport to insulin. There is evidence suggesting that activation of p38 MAP kinase (p38) is involved in the stimulation of glucose transport by insulin and contractions. Exercise results in an increase in p38 phosphorylation that lasts for hours. In this context, we tested the hypothesis that activation of p38 results in an increase in insulin sensitivity. Muscles were exposed to anisomycin for 30 min to activate p38. Anisomycin increased p38 phosphorylation ~2.5-fold and glucose transport activity 2- to 3-fold. Three hours after anisomycin treatment, by which time the acute effect of insulin on glucose transport had partially worn off, sensitivity of muscle glucose transport to 60 μU/mL insulin was markedly increased. Both the activation of p38 and the increase in insulin sensitivity induced by anisomycin were completely prevented by pretreatment of muscles with the p38 inhibitor SB-202190. However, in contrast to the finding with anisomycin, inhibition of p38 activation did not prevent the contraction-induced increase in insulin sensitivity. Thus our results show that activation of p38 is followed by an increase in insulin sensitivity of muscle glucose transport. However, activation of p38 is not necessary for induction of an increase in muscle insulin sensitivity by contractions. This finding provides evidence that contractions have an additional effect that makes p38 activation unnecessary for enhancement of insulin sensitivity by contractile activity.

mitogen-activated protein kinase; anisomycin; muscle contractions; soleus muscle; epitrochlearis muscle

EXERCISE SUCH AS RUNNING OR SWIMMING and electrical stimulation of muscles to contract induce an increase in glucose transport activity in skeletal muscle (20). This acute effect of muscle contractions on glucose transport is independent of the effect of insulin and reverses rapidly after cessation of contractile activity (20). As this increase in glucose transport wears off, it is replaced by an increase in insulin sensitivity. This phenomenon was discovered more than two decades ago by Ruderman’s group (9, 28) and has been further characterized (19), but the mechanism by which it is mediated has remained elusive. In a study in which the 2-N-4-(1-azi-2,2,2-trifluoroethyl)-[2-3H]benzoyl-1,3-bis-(D-mannos-4-yloxy)-2-propylamine (ATB-[2-3H]BMPA) exofacial photolabeling technique was used to assess GLUT4 translocation, it was found that prior exercise increased GLUT4 labeling in response to a submaximal insulin stimulus that closely paralleled the increase in glucose transport (13). We interpreted this finding as indicating that the enhanced insulin sensitivity of glucose transport is mediated by increased GLUT4 translocation. However, evidence has accumulated suggesting that, in addition to causing GLUT4 translocation, insulin increases the intrinsic glucose-transporting activity of the glucose transporters (24, 31, 33). This raises the alternative possibilities that ATBBMPA binds only to activated GLUT4 or that activation of GLUT4 increases binding, so that what we interpreted as increased translocation could actually have been due to increased binding of the photolabel to GLUT4 at the cell surface.

The concept that GLUT4 is only partially active following translocation to the cell surface is based, at least in part, on the finding that inhibition of the p38 mitogen-activated protein kinase (MAPK, p38) with SB-203580, or expression of a dominant negative p38 mutant, reduces insulin-stimulated glucose transport ~50% without affecting GLUT4 translocation (24, 31, 33). Whether or not this concept is correct is currently the subject of controversy (8, 23). However, if it is correct, it would appear that stimulation of glucose transport by insulin or contractions is a two-step process, with the first being GLUT4 translocation and the second GLUT4 activation.

Muscle contractions have been shown to result in activation of p38 (10, 30, 35). It was found by Thong et al. (34) that the increase in p38 phosphorylation induced in skeletal muscle by exercise persists for at least 3 h after cessation of exercise, which is sufficiently long for the acute effect of exercise on glucose transport to wear off. This finding, together with the possible role of p38 in GLUT4 activation (31, 32) led Thong et al. to hypothesize that activation of p38 may be involved in mediating the increase in muscle insulin sensitivity after exercise (34). In this context, the purpose of this study was to evaluate the effect of activating p38 on insulin sensitivity of glucose transport in skeletal muscle and to determine whether activation of p38 mediates the increase in insulin sensitivity induced by exercise.

METHODS

Materials. 2-Deoxy-[1,2-3H]glucose was purchased from American Radiolabeled Chemicals (St. Louis, MO). [14C]mannitol was obtained from ICN Radiochemicals (Irvine, CA). SB-202190 was purchased from Calbiochem (San Diego, CA). Anti-phospho-(Thr180/Tyr182)-p38 MAPK, total p38 MAPK, anti-phospho-cAMP response element-binding protein (CREB) (Ser133), anti-phospho-stress-activated protein kinase/c-Jun NH2-terminal kinase (JNK) (Thr183/Tyr185), and anti-phospho-extracellular signal-related kinase (ERK)1/2 MAP kinase (Thr202/Tyr204) antibodies were purchased from Cell Signaling (Beverly, MA). The horseradish peroxidase

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Hypertrophy of the epitrochlearis (0.68 ± 0.05 g) was observed, while the anisomycin-induced increase in glucose transport was modest, indicating that anisomycin stimulates GLUT4 glucose transporters (1, 14). Pretreatment with SB-202190 completely blocked the increase in p38 phosphorylation induced by anisomycin (Fig. 1A). As further evidence that p38 is activated by anisomycin, we measured phosphorylation of CREB, which is a downstream target of the p38 signaling pathway. As shown in Fig. 1B, treatment of soleus muscles with anisomycin induced an approximately twofold increase in CREB phosphorylation. The increase in CREB phosphorylation was completely blocked by pretreatment with SB-202190 (Fig. 1B).

Previous studies have shown that anisomycin also activates the stress-activated protein kinase/JNK pathway, which is closely related to the p38 MAPK pathway (16). As shown in Fig. 1C, exposure to anisomycin increased JNK phosphorylation by ~60%. However, incubation with SB-202190 had no effect on JNK phosphorylation (Fig. 1C). In addition, we measured the effect of anisomycin treatment on phosphorylation of ERK1/2. There was no significant change in phosphorylation of ERK in response to treatment with anisomycin or SB (Fig. 1D).

Anisomycin induces an increase in 2-DG transport. Incubation of muscles with 10 μM anisomycin induced a modest increase in glucose transport activity in both the soleus (0.65 ± 0.07 mmol·min⁻¹·10⁻¹ mm⁻¹ basal vs. 1.33 ± 0.08 mmol·min⁻¹·10⁻¹ mm⁻¹, anisomycin, P < 0.05) and the epichlorohydrin (0.68 ± 0.07 mmol·min⁻¹·10⁻¹ mm⁻¹ basal vs. 1.21 ± 0.32 mmol·min⁻¹·10⁻¹ mm⁻¹, P < 0.05). This finding is in keeping with earlier reports that anisomycin stimulates glucose transport in 3T3-L1 adipocytes (1, 14). In those studies, the anisomycin-induced increase in glucose transport was attributed to activation of cell surface GLUT1 glucose transporters (1, 14).

Anisomycin induces an increase in insulin sensitivity. The acute effect of anisomycin on glucose transport activity did not completely reverse during the 3.5-h recovery period. Never-
theless, it is clear that anisomycin markedly enhanced the
effect of a submaximal insulin stimulus, 60 μU/ml, on 2-DG
transport in both the soleus and epitrochlearis muscles (Fig. 2).
This increase in insulin sensitivity is evident from a compari-
son of the increases in 2-DG transport induced by insulin in the
control and anisomycin-treated muscles (Fig. 2).

Inhibition of p38 prevents the increase in insulin sensitivity
induced by anisomycin. It has been shown that inhibition of
p38 with SB-202190 causes an ~50% inhibition of the stimu-
lation of glucose transport by insulin (31–33). To determine
whether the inhibitory effect of SB-202190 on insulin action
wears off sufficiently rapidly so as not to interfere with mea-
surement of insulin sensitivity, we compared the effect of 60
μU/ml insulin on glucose transport in control muscles and
muscles treated with SB-202190 3.5 h previously. As shown in
Fig. 3, there was no difference in insulin-stimulated glucose
transport between the control muscles and those that had been
exposed to SB-202190. This finding makes it possible to inves-
tigate the effect of inhibiting p38 on the anisomycin-induced
increase in insulin sensitivity.

Soleus muscles strips were incubated with or without 10 μM SB-202190 (SB) for 60 min followed
by incubation with or without 10 μg/ml anisomycin for 30 min. Homogenates of clamp-frozen muscles were used for measurement of total p38 and phospha-p38
(P-p38; A); phospha-cAMP response element-binding protein (P-CREB; B); phospha-c-Jun NH2-terminal kinase (P-JNK; C), and phospha-extracellular
signal-related kinase (P-ERK; D). Values are means ± SE for 6–8 muscles per group. *P < 0.001, anisomycin vs. basal and vs. anisomycin + SB; +P < 0.005, anisomycin and anisomycin + SB vs. basal.
Does anisomycin induce a change in muscle glycogen levels? A previous study by Coderre et al. (3) suggested that some GLUT4 vesicles in muscle are associated with glycogen, raising the possibility that the increase in insulin sensitivity after exercise is the result of a larger available pool of free GLUT4 vesicles due to glycogen depletion. In the present study, incubation of muscles with anisomycin had no significant effect on glycogen concentration (18.9 ± 1.1 μmol glucosyl units/g, control vs. 16.1 ± 1.9 μmol glucosyl units/g, anisomycin; n = 5 muscles/group).

**Does activation of p38 mediate the contraction-induced increase in insulin sensitivity?** Stimulation of soleus muscles to contract induced an approximately threefold increase in p38 phosphorylation that was completely prevented by preincubation with SB-202190 (Fig. 5). Viewed in the context of the evidence that activation of p38 by anisomycin increases insulin sensitivity of muscle glucose transport, this finding strongly suggested that contractions may increase insulin sensitivity by activation of p38. However, in both the soleus and epitrochlearis muscles, the same stimulation protocol that increased p38 phosphorylation induced increases in insulin sensitivity despite
DISCUSSION

The novel information provided by this study is that treatment of skeletal muscles with anisomycin is followed by a dramatic increase in insulin sensitivity of the glucose transport process. Anisomycin is a potent and well-characterized activator of p38 (25, 29, 39). Our results confirm that anisomycin activates p38 in skeletal muscle and show that the increase in insulin sensitivity of glucose transport induced by anisomycin is mediated by activation of p38. The evidence that p38 mediates the anisomycin-induced increase in insulin sensitivity is provided by the finding that pretreatment of muscles with SB-202190 completely prevented the increase in insulin sensitivity. The closely related pyridimyl imidazoles SB-203580 and SB-202190 are specific inhibitors of p38 activation (5, 6). In the present study, SB-202190 blocked the increase in p38 activation by anisomycin, as evidenced by prevention of p38 phosphorylation on Thr180/Tyr182 and CREB phosphorylation on Ser133. CREB is a downstream substrate of the p38-signaling pathway (6). In addition to activating p38, anisomycin activates the JNK pathway (16), and treatment of muscles with anisomycin in the present study also resulted in increased phosphorylation of JNK. However, the anisomycin induced increase in JNK phosphorylation was not inhibited by SB-202190.

Four isoforms of p38 MAPK have been identified in skeletal muscle \( \alpha, \beta, \delta, \gamma \) (11, 21, 22, 26). The antibody used in the present study is not isoform specific; however, the SB compounds inhibit only the p38 MAPK \( \alpha \)- and \( \beta \)-isoforms (4). Because the activation of p38 MAPK with anisomycin is completely inhibited by SB-202190, it appears that the \( \alpha \)- and/or \( \beta \)-isoform(s) is/are involved in mediating the increase in insulin sensitivity seen with anisomycin.

A number of studies have provided evidence suggesting that activation of p38 mediates a step in the stimulation of glucose transport by insulin and contractions (31–33). It was found that inhibition of insulin- or contraction-stimulated activation of p38 reduced the increase in glucose transport induced by these agents by \( \sim 50\% \) without affecting GLUT4 translocation to the cell surface (31–33). These findings were interpreted as evidence that the GLUT4 translocated to the cell surface is only partially active and that a second step that involves activation of p38 by insulin or contractions is necessary to fully activate the GLUT4 (31–33). Other studies have provided evidence that argues against this concept (8, 23).

The subsequent finding that the increase in p38 phosphorylation induced in skeletal muscle by exercise persists for at
least 3 h in vivo led Thong et al. (34) to suggest that p38 may be involved in mediating the increase in muscle insulin sensitivity after exercise. On the basis of our evidence that anisomycin increases insulin sensitivity by activating p38, it appeared highly probable that this hypothesis might be correct. However, inhibition of p38 activation with SB-202190 had absolutely no effect on the increase in insulin sensitivity of glucose transport induced by contractile activity. One possibility suggested by this finding is that contractile activity and anisomycin increase muscle insulin sensitivity by different mechanisms. Another is that contractions and anisomycin activate the same signaling pathway leading to increased insulin sensitivity but that, in addition to activating p38, contractions activate another step in the pathway that is downstream of p38, thus bypassing the inhibitory effect of SB-202190. Clearly this is a fertile area for further research.

In conclusion, the results of this study provide evidence that activation of p38 is followed by an increase in the sensitivity of glucose transport to insulin in skeletal muscle. However, activation of p38 is not necessary for the enhancement of muscle insulin sensitivity by contractile activity.

ACKNOWLEDGMENTS

We thank Kathleen Hucker for excellent technical assistance and Victoria Reckamp for expert assistance in preparation of this manuscript.

GRANTS

This research was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-18986. D. C. Wright was initially supported by an American Diabetes Association Mentor-Based Postdoctoral Fellowship and subsequently by National Research Service Award AG-00078. P. C. Geiger was supported by National Research Services Award AG-00078.

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