Original Articles

Removal of Adenosine Decreases the Responsiveness of Muscle Glucose Transport to Insulin and Contractions

Dong-Ho Han, Polly A. Hansen, Lorraine A. Nolte, and John O. Holloszy

Adenosine in the extracellular space modulates stimulated glucose transport in striated muscle. In the heart and in adipocytes, adenosine potentiates insulin-stimulated glucose transport. There is controversy regarding the effect of adenosine in skeletal muscle, with reports of both an inhibitory effect and no effect, on insulin-stimulated glucose transport. We found that, in rat epitrochlearis and soleus muscles, removing adenosine with adenosine deaminase or blocking its action with the adenosine receptor blocker CPDPX markedly reduces the responsiveness of glucose transport to stimulation by *1*) insulin alone, *2*) contractions alone, and *3*) insulin and contractions in combination. Measurement of the increase in GLUT4 at the cell surface in response to a maximally effective insulin stimulus in the epitrochlearis muscle, using the exofacial label ATB-[^{3}H]BMPA, showed that adenosine deaminase treatment markedly reduces cell-surface GLUT4 labeling. The reduction in cell-surface GLUT4 labeling was similar in magnitude to the decrease in maximally insulin-stimulated glucose transport activity in adenosine deaminase–treated muscles. These results show that adenosine potentiates insulin- and contraction-stimulated glucose transport in skeletal muscle by enhancing the increase in GLUT4 at the cell surface and raise the possibility that decreased adenosine production or action could play a causative role in insulin resistance. *Diabetes* 47:1671–1675, 1998

Some of the adenosine that is produced intracellularly is released into the extracellular space, where it interacts with adenosine receptors to regulate various physiological processes in an autocrine manner (1). One such action of adenosine is to modulate insulin-stimulated glucose transport in striated muscle and adipocytes. In both adipocytes (2,3) and cardiac muscle (4–6), adenosine enhances the stimulation of glucose uptake by insulin, and removing adenosine with adenosine deaminase or blocking adenosine receptors results in a decrease in insulin-stimulated glucose uptake (2–5).

In striated muscle, glucose transport is stimulated not only by insulin but also by a separate, insulin-independent pathway activated by contractile activity (7). The maximal effects of insulin and contractions on glucose transport are additive (8–10). In perfused, beating hearts, glucose uptake is increased by adenosine in the absence of insulin, suggesting that adenosine may also potentiate the effect of contractions on glucose transport (5,6).

While the potentiating effect of adenosine on insulin-stimulated glucose transport in heart muscle and adipocytes is well documented, the literature on the role of adenosine in regulating glucose transport in skeletal muscle is confusing. In contrast with the findings in heart muscle, reports from Newsholme and colleagues (11–14) have described an inhibitory effect of adenosine on insulin-stimulated glucose uptake and metabolism in rat soleus and extensor digitorum longus muscles. In those studies, removing adenosine with adenosine deaminase or blocking its action with adenosine receptor antagonists increased muscle insulin sensitivity with no effect on insulin responsiveness (11–14). Vergauwen et al. (15), working with a perfused rat hindquarter preparation, were unable to confirm the finding of Newsholme's group that blocking adenosine receptors enhances the sensitivity of muscle glucose uptake to insulin. In contrast with the findings on heart muscle, Vergauwen et al. (15) did not find an inhibitory effect of adenosine receptor blockade on either insulin- or contraction-stimulated glucose transport. They did report, however, that adenosine receptor blockade had an inhibitory effect on the combined effect of insulin and contractions. The present study was undertaken to reinvestigate the effect of removing extracellular adenosine with adenosine deaminase or blocking adenosine receptors with the adenosine receptor antagonist CPDPX (8-cyclopentyl-1,3-dipropylxanthine) on insulin- and contraction-stimulated glucose transport in skeletal muscle.

RESEARCH DESIGN AND METHODS

Materials. 3MG (3-*O*-methyl-D-[^{3}H]glucose) and [U-^{14}C]mannitol were purchased from New England Nuclear (Boston, MA). 2DG (2-deoxy-D-[1,2-^{3}H]glucose) was obtained from American Radiolabeled Chemicals (St. Louis, MO). CPDPX and CPA (N^6-cyclopentyl-adenosine) were obtained from Research Biochemicals International (Natick, MA). Adenosine deaminase was purchased from Boehringer Mannheim (Indianapolis, IN). The exofacial label ATB-[2-^{3}H]BMPA [2-*N*-4(1-azi-2,2,2-trifluroethyl)-benzoyl-1,3-bis-(mannose-4-yloxy)-2-propylamine; specific activity ~10 Ci/mmol] was a gift of Dr. Geoffrey Holman (16). A rabbit antiserum (G4829) raised against a synthetic peptide correspond-

From the Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri.

Address correspondence and reprint requests to John O. Holloszy, MD, Division of Geriatrics and Gerontology, Washington University School of Medicine, 4566 Scott Ave., Campus Box 8113, St. Louis, MO 63110. E-mail: jhollosz@imgate.wustl.edu.

Received for publication 13 February 1998 and accepted in revised form 3 August 1998.

ATB-[2-^{3}H]BMPA, 2-*N*-4(1-azi-2,2,2-trifluroethyl)-benzoyl-1,3-bis-(mannose-4-yloxy)-2-propylamine; BSA, bovine serum albumin; CPA, N^6-cyclopentyl-adenosine; CPDPX, 8-cyclopentyl-1,3-dipropylxanthine; 2DG, 2-deoxy-D-[1,2-^{3}H]glucose; KHBB, Krebs-Henseleit bicarbonate buffer; 3MG, 3-*O*-methyl-D-[^{3}H]glucose.

g to the COOH-terminal 16 amino acids of rat GLUT4 was a gift of Dr. Mike ueckler. The cAMP assay kit was obtained from Cayman Chemical (Ann Arbor, I). Purified porcine insulin (Iletin II) was obtained from Eli Lilly (Indianapolis, J). All other reagents were purchased from Sigma (St. Louis, MO).

reatment of animals and muscle preparation. Colony-bred male Wistar its were maintained on a diet of Purina rodent chow and water. On the day before n experiment, food was restricted to 4 g per animal after 5:00 P.M. At the time of e experiment, animals weighed ~100 g. Rats were anesthetized by intraperitoneal jection of pentobarbital sodium (5 mg/100 g body wt), and epitrochlearis and oleus muscles were removed. The epitrochlearis is a small, thin muscle of the relimb, which has been shown to be suitable for in vitro measurement of glu- ose transport (17). The intact soleus muscle, which is too thick for accurate meas- ement of glucose transport in vitro, was split longitudinally into strips having n average weight of 15–20 mg (18).

luscle incubation. Immediately after dissection, muscles were placed in 2 ml xygenated Krebs-Henseleit bicarbonate buffer (KHBB) containing 8 mmol/l ucose, 32 mmol/l mannitol, and 0.1% radioimmunoassay grade bovine serum albu- in (BSA) and allowed to recover for 30 min at 30°C. Muscles were then trans- rred to 2 ml of identical medium with or without 5 U/ml adenosine deaminase, ith 1 or 70 µmol/l CPA or CPDPX, and with or without either 60 µU/ml or 2 mU/ml sulin for 30 min at 30°C. The flasks were gassed with 95% O_2, 5% CO_2 and aken continuously in a Dubnoff incubator (Precision Scientific, Chicago).

lectrical stimulation. After the initial incubations, muscles were attached to vertical Lucite rod containing two platinum electrodes and immersed in KHBB ontaining 8 mmol/l glucose, 32 mmol/l mannitol, 0.1% BSA, and the same addi- ons as the preceding incubation. The muscles were stimulated with supramax- al square-wave pulses of 0.2 ms duration with a Grass SII stimulator (Grass struments, Quincy, MA). Ten tetanic contractions were produced by stimulat- g at 100 Hz for 10 s at a rate of 1 contraction per minute for 10 min. This stim- ation protocol elicits a maximal effect on glucose transport activity (19).

leasurement of glucose transport activity. After pretreatment, muscles ere transferred to a flask containing 2 ml KHBB with 40 mmol/l mannitol and cubated with shaking for 10 min to remove glucose from the extracellular pace. Muscles were then incubated at 30°C in 1.5 ml KHBB containing 4 mmol/l DG (1.5 µCi/ml), 36 mmol/l mannitol (0.2 µCi/ml), or 8 mmol/l 3MG (2.2 µCi/ml) nd 32 mmol/l mannitol (0.2 µCi/ml), 0.1% BSA, and the same additions as the pre- ding incubation. Initial rates of 3MG transport were measured over 10 min; dur- g that time period, intracellular accumulation of 3MG is linear under the exper- ental conditions used in this study (17). 2DG uptake was measured over a 0-min period; 2DG uptake is linear for 60 min or longer under our experimental onditions (20).

hotolabeling of GLUT4 in epitrochlearis muscle. The ATB-[^{3}H]BMPA exo- cial photolabeling technique was used to measure GLUT4 at the cell surface in pitrochlearis muscle as described (21) except that the labeled GLUT4 was mmunoprecipitated with G4829.

AMP analysis. cAMP concentrations were measured with a Cayman cAMP say kit.

tatistical analysis. Data are means ± SE. Statistical analyses were performed sing Student's t test at a significant α level of $P < 0.05$.

ESULTS

ffect of adenosine deaminase on glucose transport ctivity. Addition of adenosine deaminase to the incubation nedium had no significant effect on basal 2DG uptake but narkedly decreased the effects of a maximally effective oncentration of insulin alone, of contractions alone, and of ontractions plus insulin (Fig. 1). The effect of adenosine eaminase on glucose transport activity was also examined n the soleus muscle. In the soleus, glucose transport is more nan twice as responsive to insulin as to contractions; in the pitrochlearis, insulin and contractions induce similar ncreases in glucose transport. As shown in Fig. 2, treatment vith adenosine deaminase also resulted in large decreases in ne stimulation of 2DG uptake by insulin, contractions, and nsulin plus contractions in the soleus.

Under the conditions of these experiments, 2DG can nor- nally be used to measure glucose transport (20). However, o make certain that treatment with adenosine deaminase was ot acting via an inhibition of hexokinase, we did an experi- nent in which 3MG was used to measure glucose transport ctivity. Incubation of muscle with adenosine deaminase caused a large decrease in maximally insulin-stimulated 3MG transport in epitrochlearis that was similar in magnitude to that observed when 2DG was used to measure glucose transport (3MG transport rate averaged [means ± SE] 1.27 ± 0.12 µmol · ml^{-1} · 10 min^{-1} in 12 control muscles vs. 0.75 ± 0.07 µmol · ml^{-1} · 10 min^{-1} in 12 muscles treated with adenosine deaminase, $P < 0.001$).

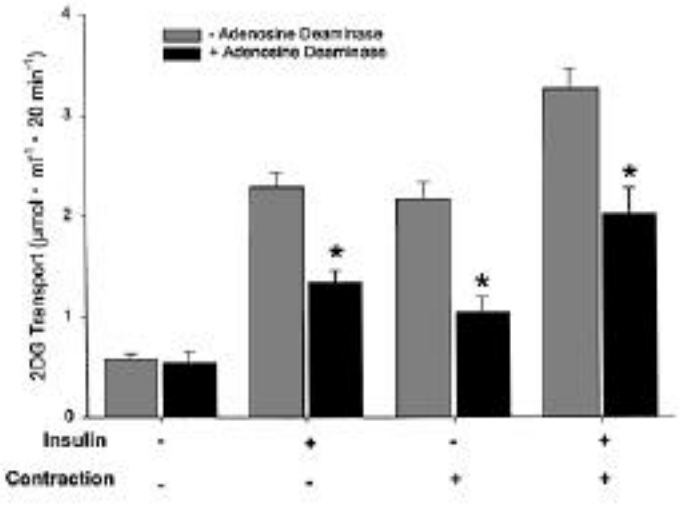

FIG. 1. Effect of adenosine deaminase on basal, maximally insulin-stimulated, contraction-stimulated, and insulin plus contraction–stimulated 2-deoxyglucose transport in epitrochlearis muscle. Values are means ± SE for 10–12 muscles per group. *Adenosine deaminase–treated versus control, $P < 0.01$.

Effect of CPA on insulin-stimulated glucose transport. To evaluate the possibility that endogenously produced adenosine was insufficient to maximally potentiate stimulated glucose transport activity, we examined the effect of adding the adenosine analog CPA to the incubation medium. A maximally effective insulin stimulus (2 mU/ml) induced an

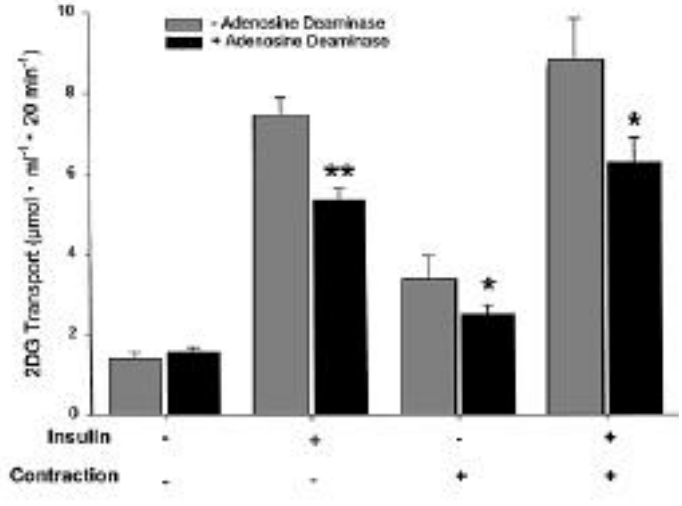

FIG. 2. Effect of adenosine deaminase on basal, maximally insulin-stimulated, contraction-stimulated, and insulin plus contraction–stimulated 2-deoxyglucose transport in soleus muscle. Values are means ± SE for 9–12 muscles per group. *Adenosine deaminase–treated versus control, $P < 0.05$.

increase in 3MG transport of 1.04 ± 0.14 μmol · ml^{-1} · 10 min^{-1} in the absence of the adenosine receptor agonist, compared with an increase of 1.03 ± 0.17 μmol · ml^{-1} · 10 min^{-1} in the presence of 1 μmol/l CPA. This finding provides evidence that the endogenously produced adenosine provides a maximally potentiating effect on stimulated transport in our muscle preparation.

Effect of CPDPX on glucose transport activity. We also examined the effect of the adenosine receptor blocker CPDPX, at a concentration similar to that used by Vergauwen et al. (15), on stimulated glucose transport. As shown in Fig. 3, incubation of epitrochlearis muscles with CPDPX resulted in a large decrease in insulin-stimulated 2DG transport (~50%), both at a maximally effective insulin concentration (2 mU/ml) and at a concentration of insulin (60 μU/ml) that produces approximately a half-maximal effect in this muscle preparation. Thus, the decrease in glucose transport activity at the lower insulin concentration was proportional to that seen at the maximally effective insulin concentration. This finding, that the increases in glucose transport activity induced by 2 mU/ml and 60 μU/ml of insulin were both reduced by ~50%, provides evidence that the entire effect of adenosine is on insulin responsiveness without a separate effect on insulin sensitivity. Contraction-stimulated glucose transport activity was similarly reduced, by ~50%, in the epitrochlearis by exposure to CPDPX. Insulin-stimulated 2DG transport was also reduced by exposure to CPDPX in the soleus, by ~30% (data not shown).

Effect of adenosine deaminase on insulin-stimulated increase in GLUT4 at the cell surface. The effect of removing adenosine with adenosine deaminase on GLUT4 at the cell surface was assessed using the exofacial label ATB-[^{3}H]BMPA. As shown in Fig. 4, treatment of muscles with adenosine deaminase resulted in a marked reduction in the magnitude of the increase in GLUT4 labeling induced by a maximally effective insulin stimulus. The decrease in cell surface GLUT4 labeling was similar in magnitude to the reduction in maximally insulin-stimulated 3MG transport caused by treatment with adenosine deaminase.

cAMP levels. One of the actions mediated by interaction of adenosine with the A_1 receptor is inhibition of adenylate cyclase activity. As a consequence, some of the metabolic effects of adenosine have been attributed to a decrease in cAMP concentration. cAMP levels were, therefore, measured in epitrochlearis muscles incubated with 2 mU/ml insulin with or without adenosine deaminase. The concentration of cAMP was low in the control muscles, and incubation with adenosine deaminase had no significant effect on cAMP level (control 126 ± 12 vs. adenosine deaminase–treated 113 ± 14 pmol/g muscle wet wt, 12 muscles per group).

DISCUSSION

The results of this study show that removing adenosine with adenosine deaminase or blocking its action with an adenosine receptor antagonist causes a large decrease in stimulated glucose transport activity in skeletal muscle. Both insulin- and contraction-stimulated glucose transport are reduced, as is the combined effect of insulin plus contractions. This decrease in stimulated glucose transport could be due to either a reduction in the number of GLUT4 at the cell surface or a decrease in GLUT4 intrinsic glucose transport activity. We found that adenosine removal with adenosine deaminase results in a reduction in the number of GLUT4 at the cell surface in maximally insulin-stimulated muscles. That decrease in GLUT4 at the cell surface closely matches the decrease in the insulin responsiveness of muscle glucose transport. This finding provides evidence that the entire effect of adenosine removal on insulin-stimulated muscle glucose transport is attributable to a reduction in the number of GLUT4 accessible to glucose or to the ATB-[^{3}H]BMPA photolabel at the cell surface.

Although there are important differences in the regulation of glucose uptake between striated muscle and adipocytes, the mechanisms involved in the stimulation of glucose transport by insulin appear to be similar in those tissues (7). It is, there-

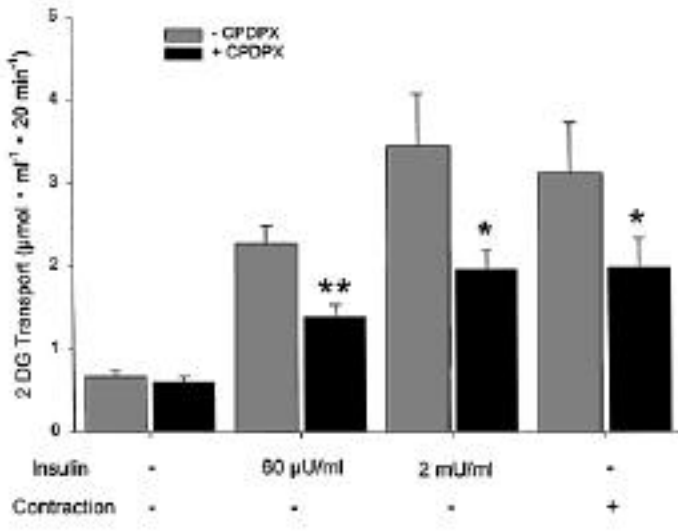

FIG. 3. Effect of CPDPX on basal, submaximally insulin-stimulated (60 μl/ml), maximally insulin stimulated (2 mU/ml), and contraction-stimulated 2-deoxyglucose transport in epitrochlearis muscle. Values are means ± SE for 6 muscles per group except the 60 μU/ml insulin group, in which there were 12 muscles. CPDPX-treated versus control: *P < 0.05, **P < 0.01.

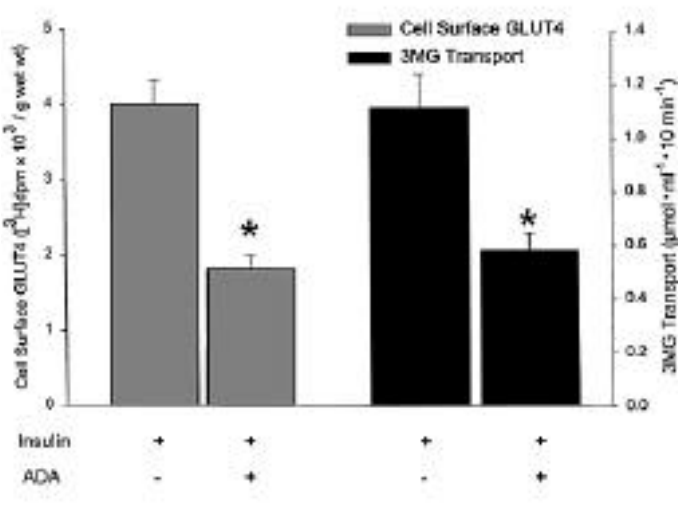

FIG. 4. Effects of a maximally effective insulin stimulus on cell surface GLUT4 and on 3MG transport in the presence or absence of adenosine deaminase. Amount of GLUT4 at the cell surface was determined with the exofacial label ATB-[^{3}H]BMPA. Values are means ± SE for six muscles per group. *Adenosine deaminase–treated versus control, P < 0.01.

ore, of interest relative to our findings that in a number of studes adenosine enhances insulin-stimulated glucose transport n fat cells (2,3,22,23). As in the present study on muscle, Furoda et al. (2) found that the potentiation of insulin-stimuated glucose transport by adenosine in adipocytes is cAMPndependent. Thus, it appears that the effects of adenosine on nsulin-stimulated glucose transport are very similar in dipocytes and skeletal muscle. Of particular interest, relative o the mechanism of action of adenosine, Vannucci et al. (3) ound that the quantity of GLUT4 measured by Western blotng was the same in the plasma membrane fraction prepared y differential centrifugation from adipocytes stimulated with nsulin in the presence and absence of adenosine. However, ell-surface GLUT4 labeling with the ATB-[^{3}H]BMPA photoabel changed in parallel with glucose transport in response o adenosine. The authors interpreted this finding to indicate hat after insulin stimulation, GLUT4 exists in two distinct tates in the adipocyte plasma membrane, one that is funconal and accessible to extracellular substrate, and one that s nonfunctional and unable to bind extracellular substrate (3). hey proposed that adenosine enhances insulin-stimulated gluose transport by promoting an "unoccluded" conformation f GLUT4 in the plasma membrane that is accessible to extraellular substrate (3).

The results of this study showing that adenosine removal ecreases insulin action are in marked contrast with the eports by Newsholme's group (11–14) that removing adenoine or blocking its action increases insulin sensitivity of nuscle glucose uptake, that is, that adenosine causes insulin esistance. We have no explanation for this discrepancy, which is made even more puzzling by the fact that some of our xperiments were done on the split soleus muscle preparaon that was also used in most of the studies by Newholme's group. However, our findings are in keeping with the vidence that adenosine potentiates the effect of insulin on lucose uptake in heart muscle (4–6) and adipocytes 2,3,22,23). The finding that adenosine also increases gluose uptake in beating hearts in the absence of insulin (5,6) uggests that, as we found in skeletal muscle, adenosine otentiates the effect of contractile activity on glucose transort in cardiac muscle.

Vergauwen et al. (15) studied the effect of adenosine eceptor blockers in a perfused rat hindquarter preparaon. Unlike Newsholme's group, they found no evidence that denosine inhibits either insulin-stimulated or contractiontimulated glucose uptake. In contrast to our findings on isoated muscles, they observed no inhibitory effect of the denosine receptor blocker CPDPX on the stimulation of lucose uptake by either insulin alone or contractions lone. They did, however, find that adenosine receptor lockade resulted in a decrease in the stimulation of glucose ptake by insulin and contractions in combination (15). he reason for their inability to detect the inhibitory effect f adenosine receptor blockade on insulin- or contractiontimulated muscle glucose transport may relate to the xperimental model used. Although the perfused hindlimb nodel has provided important insights regarding the regulation of muscle glucose uptake (24), it is not ideal for evalating the effects of inhibitors, because in perfused muscle, r muscle in vivo, either muscle cell membrane permeabily to glucose or glucose availability can limit the rate of gluose uptake (25,26).

Adenosine deaminase or CPDPX markedly reduced stimulated glucose transport activity in both epitrochlearis and soleus muscles in the present study. The soleus consists predominantly of type I slow-twitch fibers (27), while the epitrochlearis consists almost entirely of type II (mostly type IIb), fast-twitch fibers (28). Thus, our results show that adenosine potentiates the effects of contractions and insulin in both fast-twitch and slow-twitch muscle fibers. Vergauwen et al. (15), however, reported that blocking adenosine receptors with caffeine caused a 42% decrease in 3MG uptake stimulated by insulin plus contractions only in the slow-twitch red fibers, with no significant effect on fast-twitch red (type IIa) or fast-twitch white (type IIb) fibers. This finding led them to conclude that the effect of blocking adenosine receptors on glucose uptake in contracting muscles perfused with insulin is limited to slow-twitch red fibers. They also reported, however, that hindquarter glucose uptake under those conditions was reduced ~25%. It can be calculated from the glucose transport rates reported for the different fiber types by Vergauwen et al. (15) and the evidence that slow-twitch red fibers make up only ~5% of the total muscle mass in rat hindlimbs (27) that the decrease in insulin plus contraction–stimulated glucose uptake caused by CPDPX or caffeine would have been just ~4% if the effect was, in fact, only on type I fibers.

Contractions and insulin stimulate muscle glucose transport via separate pathways. This is evidenced by the findings that *1*) the maximal effects of insulin and contractions on both glucose transport (7) and the increase in GLUT4 at the cell surface (29,30) are additive, and *2*) it is possible to completely inhibit insulin-stimulated glucose transport without affecting contraction-stimulated glucose transport (30–32). Our results show that adenosine acts on both contraction-stimulated and insulin-stimulated glucose transport. This finding provides evidence that the potentiating effect of adenosine on stimulated glucose transport in muscle occurs at a common step beyond the contraction and insulin signaling pathways, thus pointing the direction for future studies on the mechanism of adenosine action.

In conclusion, the results of this study show that removing adenosine with adenosine deaminase or blocking its action with CPDPX markedly reduces the responsiveness of muscle glucose transport to stimulation by insulin, by contractions, and by insulin and contractions together. The decrease in insulin-stimulated glucose transport is mediated by a smaller increase in GLUT4 at the cell surface. The decrease in stimulated glucose transport induced by removing adenosine or blocking its action occurs in both fast-twitch and slow-twitch muscle. These findings raise the possibility that decreased adenosine production or action could play a role in the development of insulin resistance.

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health Grant DK-18986. D.H.H. was supported initially by Institutional National Research Service Award AG-00078 and subsequently by a mentor-based fellowship from the American Diabetes Association. L.A.N. was supported by Institutional National Research Service Award AG-00078.

We thank Vanessa Kieu and May Chen for excellent technical assistance and Victoria Reckamp for expert assistance in preparation of the manuscript.

REFERENCES

1. Stiles GL: Adenosine receptors. *J Biol Chem* 267:6451–6454, 1992
2. Kuroda M, Honnor RC, Cushman SW, Londos C, Simpson IA: Regulation of insulin-stimulated glucose transport in the isolated rat adipocyte. *J Biol Chem* 262:245–253, 1987
3. Vannucci SJ, Nishimura H, Satoh S, Cushman SW, Holman GD, Simpson IA: Cell surface accessibility of GLUT4 glucose transporters in insulin-stimulated rat adipose cells. *Biochem J* 288:325–330, 1992
4. Law WR, Raymond RM: Adenosine potentiates insulin-stimulated myocardial glucose uptake in vivo. *Am J Physiol* 254:H970–H975, 1988
5. Wyatt DA, Edmunds MC, Rubio R, Berne RM, Lasley RD, Mentzer RM Jr: Adenosine stimulates glycolytic flux in isolated perfused rat hearts by A1-adenosine receptors. *Am J Physiol* 257:H1952–H1957, 1989
6. Angello DA, Berne RM, Coddington NM: Adenosine and insulin mediate glucose uptake in normoxic rat hearts by different mechanisms. *Am J Physiol* 265:H880–H885, 1993
7. Holloszy JO, Hansen PA: Regulation of glucose transport into skeletal muscle. In *Reviews of Physiology, Biochemistry and Pharmacology*. Blaustein MP, Grunicke H, Habermann E, Pette D, Schultz G, Schweiger M, Eds. Berlin, Springer-Verlag, 1996, p. 99–193
8. Nesher R, Karl IE, Kipnis DM: Dissociation of effects of insulin and contraction on glucose transport in rat epitrochlearis muscle. *Am J Physiol* 249:C226–C232, 1985
9. Zorzano A, Balon TW, Goodman MN, Ruderman NB: Additive effects of prior exercise and insulin on glucose and AIB uptake by muscle. *Am J Physiol* 251:E21–E26, 1986
10. Constable SH, Favier RJ, Cartee GD, Young DA, Holloszy JO: Muscle glucose transport: Interactions of in vitro contractions, insulin and exercise. *J Appl Physiol* 64:2329–2332, 1988
11. Budohoski L, Challiss RAJ, McManus B, Newsholme EA: Effects of analogues of adenosine and methyl xanthines on insulin sensitivity in soleus muscle of the rat. *FEBS Lett* 167:1–4, 1984
12. Espinal J, Challiss RAJ, Newsholme EA: Effect of adenosine deaminase and an adenosine analogue on insulin sensitivity in soleus muscle of the rat. *FEBS Lett* 158:103–106, 1983
13. Leighton B, Lozeman FJ, Vlachonikolis IG, Challiss RAJ, Pitcher JA, Newsholme EA: Effects of adenosine deaminase on the sensitivity of glucose transport, glycolysis and glycogen synthesis to insulin in muscles of the rat. *Int J Biochem* 20:23–27, 1988
14. Challiss RAJ, Budohoski L, McManus B, Newsholme EA: Effects of an adenosine-receptor antagonist on insulin-resistance in soleus muscle from obese Zucker rats. *Biochem J* 221:915–917, 1984
15. Vergauwen L, Hespel P, Richter EA: Adenosine receptors mediate synergistic stimulation of glucose uptake and transport by insulin and by contractions in rat skeletal muscle. *J Clin Invest* 93:974–981, 1994
16. Holman GD, Kozka IJ, Clark AE, Flower CJ, Saltis J, Habberfield AD, Simpson IA, Cushman SW: Cell surface labeling of glucose transporter isoform GLUT4 by bis-mannose photolabel. *J Biol Chem* 265:18172–18179, 1990
17. Young DA, Uhl JJ, Cartee GD, Holloszy JO: Activation of glucose transport in muscle by prolonged exposure to insulin: effects of glucose and insulin concentration. *J Biol Chem* 261:16049–16053, 1986
18. Henriksen EJ, Holloszy JO: Effect of diffusion distance on measurement of rat skeletal muscle glucose transport in vitro. *Acta Physiol Scand* 143:381–386, 1991
19. Henriksen EJ, Bourey RE, Rodnick KJ, Koranyi L, Permutt MA, Holloszy JO: Glucose transporter protein content and glucose transport capacity in rat skeletal muscles. *Am J Physiol* 259:E593–E598, 1990
20. Hansen PA, Gulve EA, Holloszy JO: Suitability of 2-deoxyglucose for in vitro measurement of glucose transport activity in skeletal muscle. *J Appl Physiol* 76:979–985, 1994
21. Hansen PA, Corbett JA, Holloszy JO: Phorbol esters stimulate muscle glucose transport by a mechanism distinct from the insulin and hypoxia pathways. *Am J Physiol* 273:E28–E36, 1997
22. Taylor WM, Halperin ML: Stimulation of glucose transport in rat adipocytes by insulin, adenosine, nicotinic acid and hydrogen peroxide. *Biochem J* 178:381–389, 1979
23. Joost H-G, Steinfelder H-J: Modulation of insulin sensitivity by adenosine. Effects on glucose transport, lipid synthesis, and insulin receptors of the adipocyte. *Mol Pharmacol* 22:614–618, 1982
24. Bonen A, Clark MG, Henriksen EJ: Experimental approaches in muscle metabolism: hindlimb perfusion and isolated muscle incubations. *Am J Physiol* 266:E1–E16, 1994
25. Hespel P, Vergauwen L, Vandenberghe K, Richter EA: Important role of insulin and flow in stimulating glucose uptake in contracting skeletal muscle. *Diabetes* 44:210–215, 1995
26. Holmang A, Mimura K, Bjorntorp P, Lonnroth P: Interstitial muscle insulin and glucose levels in normal and insulin-resistant Zucker rats. *Diabetes* 46:1799–1804, 1997
27. Armstrong RB, Phelps RO: Muscle fiber type composition of the rat hindlimb. *Am J Anat* 171:259–272, 1984
28. Nesher R, Karl IE, Kaiser KE, Kipnis DM: Epitrochlearis muscle. I. Mechanical performance, energetics, and fiber composition. *Am J Physiol* 239:E454–E460, 1980
29. Gao J, Ren J, Gulve EA, Holloszy JO: Additive effect of contractions and insulin on GLUT4 translocation into the sarcolemma. *J Appl Physiol* 77:1597–1601, 1994
30. Lund S, Holman GD, Schmitz O, Pedersen O: Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through mechanism distinct from that of insulin. *Proc Natl Acad Sci U S A* 92:5817–5821, 1995
31. Lee AD, Hansen PA, Holloszy JO: Wortmannin inhibits insulin-stimulated but not contraction-stimulated glucose transport activity in skeletal muscle. *FEBS Lett* 361:51–54, 1995
32. Yeh J-I, Gulve EA, Rameh L, Birnbaum MJ: The effects of wortmannin in rat skeletal muscle. Dissociation of signaling pathways for insulin- and contraction-activated hexose transport. *J Biol Chem* 270:2107–2111, 1995

Author Queries (please see Q in margin and underlined text)

Q1:Please supply name and location of manufacturer of incubator.
Q2:Please supply name and location of manufacturer of stimulator.