Propranolol prevents epinephrine from limiting insulin-stimulated muscle glucose uptake during contraction

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Hunt, Desmond G., Zhenping Ding, and John L. Ivy. Propranolol prevents epinephrine from limiting insulin-stimulated muscle glucose uptake during contraction. J Appl Physiol 93: 697–704, 2002. First published April 26, 2002; 10.1152/japplphysiol.00017.2002.—β-Blockade results in rapid glucose clearance and premature fatigue during exercise. To investigate the cause of this increased glucose clearance, we studied the acute effects of propranolol on insulin-stimulated muscle glucose uptake during contraction in the presence of epinephrine with an isolated rat muscle preparation. Glucose uptake increased in both fast- (epitrochlearis) and slow-twitch (soleus) muscle during insulin or contraction stimulation. In the presence of 24 nM epinephrine, glucose uptake during contraction was completely suppressed when insulin was present. This suppression of glucose uptake by epinephrine was accompanied by a decrease in insulin receptor substrate (IRS)-1-phosphatidylinositol 3 (PI3)-kinase activity. Propranolol had no direct effect on insulin-stimulated glucose uptake during contraction. However, epinephrine was ineffective in attenuating insulin-stimulated glucose uptake during contraction in the presence of propranolol. This ineffectiveness of epinephrine to suppress insulin-stimulated glucose uptake during contraction occurred in conjunction with its inability to completely suppress IRS-1-PI3-kinase activity. Results of this study indicate that the effectiveness of epinephrine to inhibit insulin-stimulated glucose uptake during contraction is severely diminished in muscle exposed to propranolol. Thus the increase in glucose clearance and premature fatigue associated with β-blockade could result from the inability of epinephrine to attenuate insulin-stimulated muscle glucose uptake.

TWO POTENT STIMULATORS of glucose uptake in skeletal muscle are insulin and contraction (1, 7, 16, 38). Insulin stimulates glucose uptake through a complex array of intracellular signaling events. Insulin binding to the insulin receptor leads to the activation of insulin receptor tyrosine kinase and insulin receptor substrate (IRS)-1. Activated IRS-1 protein binds to a number of proteins with the Src-homology-2 domain, such as phosphatidylinositol 3-kinase (PI3-kinase). Evidence suggests that the IRS-1-PI3-kinase pathway is important in the stimulation of glucose uptake in skeletal muscle (40). At present, the mechanism by which muscle contraction stimulates glucose uptake is unknown. However, a rise in calcium concentration (41, 44), the release of autocrine and/or paracrine factors (23, 36, 42), and protein kinases, like protein kinase C and AMP-activated protein kinase, are proposed to be involved in the signal leading to the activation of glucose uptake (21, 22).

Modulating the actions of insulin and muscle contraction and controlling glucose metabolism is epinephrine (4, 19, 25, 27, 33, 35). During exercise, increased sympathetic activity increases plasma epinephrine levels and lowers plasma insulin levels (39). These hormonal changes help maintain plasma glucose levels during exercise by increasing hepatic glucose output and reducing glucose utilization (27, 35, 39). However, in individuals who are taking β-adrenergic antagonists (β-blockers) for clinical conditions such as coronary heart disease or hypertension, the ability to regulate plasma glucose is impaired during exercise. This impairment in the regulation of plasma glucose by β-adrenergic receptor blockade (β-blockade) during exercise can lead to hypoglycemia (9, 10, 14) as well as a reduction in aerobic endurance capacity (5, 8, 9). Propranolol, a β-adrenergic antagonist, increases hepatic glucose output and whole body glucose clearance during exercise (2, 19, 20, 27, 34, 35). It has been suggested that the increase in glucose clearance during exercise induced by β-blockade is due to an increased glucose utilization by the exercising musculature. However, the mechanism by which glucose utilization is increased in exercising skeletal muscle during β-blockade has not been addressed.

In a previous study from our laboratory, we demonstrated that downregulation or acute blockade of β-adrenergic receptors prevented epinephrine from antagonizing insulin-stimulated glucose uptake (15). Thus, on the basis of these results, we hypothesized that the increase in glucose utilization during exercise induced by β-blockade is due to the inability of epinephrine to attenuate muscle glucose uptake during contraction in...
the presence of insulin. Results of this study indicate that the effectiveness of epinephrine to inhibit insulin-stimulated muscle glucose uptake during contraction is severely diminished during β-blockade. Furthermore, this attenuation in the action of epinephrine was accompanied by an enhanced activation of the insulin-signaling protein PI3-kinase.

**METHODS**

**Animals and muscle preparation.** Female Sprague-Dawley rats (n = 90) between 110 and 120 g were randomly assigned to the following seven treatment groups: basal, insulin, contraction, contraction-insulin, contraction-insulin-epinephrine, contraction-insulin-propranolol, and contraction-insulin-epinephrine-propranolol. All animals were obtained from and housed in the Animal Resource Center, University of Texas at Austin, Austin, TX. The temperature of the animal room was maintained at 21°C, and a 12:12-h light-dark cycle was set. All procedures were approved by the Animal Care and Use Committee of the University of Texas and conformed to the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (DHHS Publication No. (NIH) 85-23, revised 1985, Office of Science and Health Reports, Bethesda, MD 20892).

Rats were anesthetized after an 8-h fast via an intraperitoneal injection of pentobarbital sodium (6.5 mg/100 g body wt), and the epitrochlearis (fast-twitch) and soleus (slow-twitch) muscles were then excised. The soleus muscle was separated into strips weighing ~15 mg, and the epitrochlearis was used to assess glucose uptake, glycogen, glucose 6-phosphate (G-6-P), and IRS-1-PI 3-kinase activity after in vitro incubation under the treatments previously outlined.

**Muscle incubation.** After isolation, epitrochlearis and soleus muscles were individually pinned to the contraction apparatus and preincubated for 50 min at 29°C in 3 ml of continuously gassed (95% O2-5% CO2) Krebs-Henseleit bicarbonate buffer containing 0.1% BSA, 32 mM mannitol, and 8 mM glucose. After the preincubation, muscles were washed for 10 min in fresh buffer (3 ml) containing 0.1% BSA and 40 mM mannitol. Muscles were then transferred to fresh buffer, and glucose uptake was measured in the presence of 2 mM pyruvate, 6 mM glucose, 280 μCi/ml 2-[3H]deoxy-o-glucose (2-DG Dupont, NEN, Boston, MA), 32 mM mannitol, and 8 mM glucose. The concentration of epinephrine used was selected because it is representative of a physiological concentration observed in the rat (31). The insulin concentration used was selected because it is representative of a normal physiological concentration. In addition, we previously established that 50 μU/ml insulin elicited a glucose uptake rate significantly above basal and that a clear effect of epinephrine on glucose uptake could be detected (15).

**Muscle processing for determination of glucose uptake.** Glucose uptake was estimated by determining the incorporation rate of 2-DG into skeletal muscle. 2-DG is a glucose analog that has uptake rates similar to glucose but not completely oxidized, and thus it provides a good estimate of the rate of glucose uptake in skeletal muscle. Incubated muscles were weighed and dissolved in 1 M KOH for 15 min at 60°C. Dissolved samples were then neutralized with 1 M HCl, and 0.3 ml of each supernatant was added to 6 ml of Biosafe II scintillation fluid (Research Products International, Mt. Prospect, IL). Samples were counted for 3H and 14C in an LS-6000 liquid scintillation spectrophotometer (Beckman, Fullerton, CA).

**Muscle glycogen determination.** Muscle glycogen concentration was determined after complete enzymatic degradation to glucose with amyloglucosidase (30). An aliquot of the KOH-digested muscle was incubated overnight in 0.3 M sodium acetate buffer, pH 4.8, that contained 5 mg/ml amyloglucosidase (Boehringer Mannheim, Mannheim, Germany). Liberated glucose was then measured by using a spectrophotometric Trinder reaction (Sigma Chemical).

**G-6-P determination.** Muscle samples were added to 300 μl of 10% perchloric acid and homogenized at 0°C.
RESULTS

Insulin (50 μU/ml) significantly increased glucose uptake above basal in the epitrochlearis (52%) and in the soleus (100%) (Figs. 2 and 3). Contraction stimulation increased glucose uptake in the epitrochlearis by 88% above basal, whereas there was no significant increase observed in the soleus. The simultaneous activation of glucose uptake by insulin and contraction produced glucose uptake values that were 100 and 18% greater than insulin stimulation in the epitrochlearis and soleus, respectively. Because contraction alone had no effect on glucose uptake in the soleus, contraction appeared to augment insulin-stimulated glucose uptake. Epinephrine attenuated insulin-stimulated glucose uptake during contraction in the epitrochlearis and soleus to values similar to contraction stimulation alone. This attenuation in glucose uptake by epinephrine was blocked with the β-adrenergic antagonist propranolol.

Insulin stimulation increased glycogen concentration 64 and 28% above basal in the epitrochlearis and soleus (Figs. 4 and 5). Contraction caused no significant reduction in glycogen concentration in the epitrochlearis or soleus muscles compared with basal levels. However, contraction did prevent insulin-stimulated glycogen storage. The effect of contraction was enhanced by epinephrine as glycogen levels declined significantly below basal levels in both muscles. During contraction, propranolol had no effect on muscle glycogen but was able to abolish the ability of epinephrine to stimulate glycogenolysis.

After 30 min of incubation, insulin had increased G-6-P by 77% above basal in the epitrochlearis and by 177% above basal in the soleus (Table 1). Contraction resulted in G-6-P levels that were similar to basal. Contraction plus insulin stimulation produced G-6-P concentrations that were 56 and 52% lower than insulin stimulation in the epitrochlearis and soleus, respec-
tively. In the presence of epinephrine, contraction plus insulin had no effect on the G-6-P concentration in the epitrochlearis but significantly increased G-6-P concentration above basal in the soleus. This effect of epinephrine in the soleus was blocked by propranolol.

Because G-6-P was not substantially increased after 30 min of muscle contraction, we investigated the possibility of G-6-P being increased during an earlier phase of the incubation protocol. Muscles were incubated as before except that the incubation time was reduced to 15 min (Table 1). For the epitrochlearis, G-6-P was essentially the same after 15 min of incubation with insulin as was found after 30 min of incubation. For the soleus, G-6-P was actually lower after 15 min compared with 30 min of incubation. Fifteen minutes of contraction increased G-6-P slightly, but not statistically, in both the epitrochlearis and soleus. There was no further increase in G-6-P when contraction was performed in the presence of insulin. In the presence of insulin and epinephrine, epitrochlearis G-6-P was higher after 15 min of contraction compared with 30 min of contraction, but the G-6-P concentration did not exceed the concentrations produced by 15 min of contraction or insulin stimulation alone. The soleus G-6-P concentration was similar after 15 and 30 min of contraction in the presence of insulin and epinephrine.

Insulin stimulation significantly increased IRS-1-PI3-kinase activity by 220% in the epitrochlearis and by 277% in the soleus (Figs. 6 and 7). Contraction had no effect on IRS-1-PI3-kinase activity in the epitrochlearis or soleus. Contraction reduced the ability of insulin to activate IRS-1-PI3-kinase by 14% in the epitrochlearis and by 50% in the soleus. In the presence of epinephrine, insulin-stimulated IRS-1-PI3-ki-

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**Fig. 3.** Glucose uptake in the soleus muscle. Values are means ± SE. *Significant difference from basal (P < 0.05). †Significant difference from insulin (P < 0.05). §§Significant difference from contraction (P < 0.05). #Significant difference from contraction and insulin (P < 0.05). ¶Significant difference from contraction, insulin, and epinephrine (P < 0.05).

**Fig. 4.** Glycogen in the epitrochlearis muscle. Values are means ± SE. *Significant difference from basal (P < 0.05). †Significant difference from insulin (P < 0.05). §§Significant difference from contraction (P < 0.05). #Significant difference from contraction and insulin (P < 0.05). ¶Significant difference from contraction, insulin, and epinephrine (P < 0.05).
nase activity was completely eliminated during contraction. Propranolol prevented the attenuation in IRS-1-PI3-kinase activity by epinephrine in the epitrochlearis. However, in the soleus, the effect of propranolol was less clear because of the inability of insulin to significantly activate IRS-1-PI3-kinase activity during contraction.

**DISCUSSION**

For individuals taking β-blockers for clinical conditions, the ability to regulate plasma glucose is impaired during exercise. This impairment in the regulation of plasma glucose during β-blockade and exercise can lead to hypoglycemia (9, 10, 14) as well as a reduction in aerobic endurance capacity (5, 8, 9). Propranolol administration has been shown to increase the rate of whole body glucose clearance during exercise (2, 34, 35). Thus it has been suggested that the increase in glucose clearance induced by β-blockers during exercise is due to an increase in muscle glucose utilization. However, the mechanism by which β-blockade increases muscle glucose utilization during exercise is unknown. One possibility that has been suggested is that β-blockade restricts lipolysis and reduces plasma free fatty acid availability during exercise, which results in an increased reliance on plasma glucose. However, Mora-Rodriguez et al. (28) found that, even when plasma free fatty acid levels were restored to normal exercise levels, glucose clearance, although lowered, remained significantly elevated above control, which suggests that part of the effect of β-blockade on muscle glucose uptake was independent of fat metabolism.

In the present study, we found that a physiological concentration of epinephrine could reduce muscle glucose uptake and increase glycogenolysis during contraction when insulin was present. Because we did not investigate the effect of epinephrine on contraction-stimulated glucose uptake in the absence of insulin, we cannot say for certain that the effect of epinephrine on insulin- and contraction-stimulated glucose uptake was due solely to an attenuation of insulin action. However, there are several reasons why this is the most feasible explanation. Several studies (3, 32) have found either a slight increase or no difference in contraction-stimulated glucose uptake in the presence of epinephrine. When epinephrine has been found to attenuate contraction-stimulated glucose uptake, the effect has been small compared with the effect on insu-

**Table 1. Glucose 6-phosphate concentration in epitrochlearis and soleus muscles incubated for 15 or 30 min**

<table>
<thead>
<tr>
<th>Glucose 6-Phosphate, μmol/g</th>
<th>Epitrochlearis</th>
<th>Soleus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Basal</td>
<td>0.090 ± 0.011(9)</td>
<td>0.089 ± 0.005(12)</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.169 ± 0.019(6)</td>
<td>0.157 ± 0.017(6)</td>
</tr>
<tr>
<td>Contraction</td>
<td>0.135 ± 0.029(6)</td>
<td>0.098 ± 0.016(8)</td>
</tr>
<tr>
<td>Con-Ins</td>
<td>0.118 ± 0.014(7)</td>
<td>0.068 ± 0.010(8)</td>
</tr>
<tr>
<td>Con-Ins-Epi</td>
<td>0.161 ± 0.022(6)</td>
<td>0.069 ± 0.013(7)</td>
</tr>
<tr>
<td>Con-Ins-Pro</td>
<td>0.100 ± 0.006(6)</td>
<td>0.087 ± 0.008(8)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses are number of observations. Con-Ins, contraction and insulin; Con-Ins-Epi, contraction, insulin, and epinephrine; Con-Ins-Pro, contraction, insulin, and propranolol; Con-Ins-Pro-Epi, contraction, insulin, propranolol, and epinephrine. *Significantly different from basal (P < 0.05). †Significantly different from insulin (P < 0.05). §Significantly different from contraction (P < 0.05). ¶Significantly different from 30-min corresponding mean (P < 0.05).

Fig. 5. Glycogen in the soleus muscle. Values are means ± SE. *Significant difference from basal (P < 0.05). †Significant difference from insulin (P < 0.05). §Significant difference from contraction (P < 0.05). ¶Significant difference from contraction, insulin, and epinephrine (P < 0.05).
lin-stimulated glucose uptake (1, 29). In addition, we observed that muscle glucose uptake was almost identical during contraction in the absence or presence of insulin and/or epinephrine.

This is the first study to our knowledge to demonstrate that a physiological concentration of epinephrine will suppress in vitro insulin-stimulated glucose uptake during contraction and to imply that small changes in plasma epinephrine may have a major effect on glucose utilization during exercise. However, epinephrine had no effect on insulin-stimulated glucose uptake or glycogenolysis during contraction in the presence of propranolol. It should be noted that propranolol had no effect on insulin- and contraction-stimulated glucose uptake in the absence of epinephrine, which indicates that β-blockers do not have an augmenting effect on glucose uptake per se. This finding, therefore, supports our hypothesis that the increase in muscle glucose utilization induced by β-blockade during exercise is due to the inability of epinephrine to limit skeletal muscle glucose uptake.

Several mechanisms have been proposed to explain the antagonistic effects of epinephrine on glucose uptake. Epinephrine is a known activator of glycogenolysis in skeletal muscle (4, 17, 18). The increase in glycogenolysis induced by epinephrine leads to the accumulation of the intracellular metabolite G-6-P, which inhibits hexokinase activity and glucose phosphorylation (13). Under conditions of rapid glucose transport, such as during insulin and contraction stimulation, inhibition of hexokinase by epinephrine would result in an increase in intracellular free glucose, which would cause an increase in the countertransport of glucose from the cell and reduce the rate of glucose clearance from the extracellular medium.
We found that epinephrine had a stimulating effect on glycogenolysis during insulin and contraction stimulation, thus corroborating previous findings (1). However, despite detectable glycogenolysis during insulin and contraction stimulation, epinephrine did not increase the intracellular concentration of G-6-P either in the early or late phases of incubation to levels known to attenuate hexokinase activity in skeletal muscle. This finding is inconsistent with results by Aselsen and Jensen (1) in which an increase in G-6-P was observed during contraction in the presence of insulin and epinephrine. However, in their study, Aselsen and Jensen used insulin and epinephrine concentrations that were beyond the physiological range and a higher contraction frequency than in the present study. Therefore, the differences observed in the intracellular G-6-P concentration between studies most likely resulted from the differences in experimental conditions. More importantly, the present results indicate that the inhibition of glucose uptake by epinephrine must occur through a G-6-P-independent mechanism. However, this may not mean that the inhibition of hexokinase is not responsible for the attenuation of insulin-stimulated glucose uptake by epinephrine. Walaas (37) found that epinephrine had an inhibitory effect on hexokinase activity independent of G-6-P, and Ekman and Nilsson (6) demonstrated that hexokinase could be inhibited by protein kinase A phosphorylation. Protein kinase A is activated by cAMP, the primary second messenger of epinephrine.

It is also possible that epinephrine reduces insulin-stimulated glucose uptake during contraction by reducing the activity of key insulin signaling proteins involved in glucose transport, such as IRS-1 and PI3-kinase (24). IRS-1-PI3-kinase activity was found to be significantly increased by a submaximally stimulating concentration of insulin, but this increase in IRS-1-PI3-kinase activity was partially attenuated by muscle contraction. When epinephrine and contraction were combined, however, activation of IRS-1-PI3-kinase was completely abolished. Previous studies have demonstrated that contraction will inhibit insulin-stimulated IRS-1-PI3-kinase activity (11, 40), but there have been no studies to address the combined effect of epinephrine and contraction on insulin-stimulated IRS-1-PI3-kinase activity. The present results indicate that the combination of epinephrine and contraction is a potent inhibitor of IRS-1-PI3-kinase activation by insulin. It should be noted that, in the presence of propranolol, the effect of epinephrine on insulin-stimulated IRS-1-PI3-kinase activity during contraction was significantly attenuated. Thus it is possible that the increase in muscle glucose uptake during β-blockade is due to an augmented activity of the insulin-signaling pathway.

We also observed full additivity of insulin/contraction-stimulated glucose uptake although the activation of PI3-kinase was significantly attenuated by contraction. One interpretation of these results is that full activation of IRS-1-PI3-kinase by insulin is not required for normal glucose uptake during muscle contraction. Another possibility is that full activation does not have to be maintained for normal glucose uptake during muscle contraction.

Finally, our finding that epinephrine can attenuate the activation of PI3-kinase by insulin suggests that epinephrine may be able to modulate insulin-stimulated muscle glucose transport. Although there are a few studies that support this possibility (12, 43), the majority of studies indicate that the glucose transport process is not directly regulated by epinephrine in skeletal muscle (1, 4, 25). However, an effect of epinephrine on the glucose transport process may be condition specific and may not be readily detectable.

In summary, we used an isolated muscle preparation to investigate the mechanism by which β-blockade increases glucose utilization in skeletal muscle during contraction. During exercise, both insulin and epinephrine have strong independent but contrasting effects on muscle glucose uptake and metabolism. In patients taking β-blockers, the ability to regulate plasma glucose is impaired during exercise. This impairment has been attributed to an increase in glucose utilization by exercising skeletal muscle. In the present study, propranolol was found not to have an effect on muscle glucose uptake during contraction in the presence of insulin. However, propranolol was able to prevent epinephrine from attenuating insulin-stimulated muscle glucose uptake during contraction. Epinephrine was also unable to attenuate insulin-stimulated IRS-1-PI3-kinase activity during contraction in the presence of propranolol. Thus it appears that the increase in muscle glucose uptake in vivo during β-blockade may be caused, in part, by the ineffectiveness of epinephrine to attenuate the insulin-signaling pathway.

REFERENCES


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