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Evidence for the regulation of contraction-induced fatty acid oxidation via extracellular signal-regulated kinase 1/2 activation independent of changes in fatty acid uptake

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Abstract

Data show that extracellular signal-regulated kinase 1/2 (ERK1/2) may be involved in the regulation of fatty acid (FA) uptake during muscle contraction via stimulation of CD36 translocation to the plasma membrane. The perfused hind limb model was used to determine (1) the importance of ERK1/2 signaling on contraction-induced FA uptake and (2) the effect of ERK1/2-mediated FA uptake on contraction-induced FA oxidation. We perfused rat hind limbs with 8 mmol/L glucose, 550 μmol/L palmitate, and no insulin at rest in the absence of inhibitor and during moderate-intensity electrical stimulation and dose-dependent pharmacologic inhibition of ERK1/2 using increasing concentrations of PD98059 (P1 = none, P2 = 10 μmol/L, P3 = 20 μmol/L, P4 = 50 μmol/L). Increasing PD98059 concentration resulted in a gradual decrease in contraction-induced ERK1/2 phosphorylation, and this was accompanied by a decrease in contraction-induced FA uptake (concentration required for 50% inhibition [IC_{50}] = 15.8 ± 1.6 μmol/L) and in plasma membrane CD36 content (IC_{50} = 8.7 ± 0.3 μmol/L) ($P < .05$). Percent FA oxidation was significantly lower in P3 and P4 compared with P1 and P2. Based on IC_{50} values, FA oxidation demonstrated a greater sensitivity than FA uptake to changes in ERK1/2 phosphorylation (IC_{50} = 5.4 ± 0.3 μmol/L) ($P < .05$). A positive correlation was found between FA uptake and plasma membrane CD36 content (R^2 = 0.85, $P < .05$). Plasma membrane CD36 content, FA uptake, and FA oxidation each shared a positive correlation with ERK1/2 phosphorylation (R^2 = 0.64, 0.66, and 0.71, respectively; $P < .05$). These results suggest that during moderate-intensity muscle contraction, ERK1/2 phosphorylation is required for translocation of CD36 to the plasma membrane and the subsequent increase in FA uptake. In addition, these data suggest that ERK1/2 signaling may be involved in the regulation of FA oxidation independently of its effects on FA uptake.

1. Introduction

Muscle contraction increases fatty acid (FA) uptake and oxidation in skeletal muscle [1-4]. However, the signaling mechanisms regulating the contraction-induced increase in FA metabolism are relatively unknown. It is now generally accepted that contraction-induced FA uptake is mediated in part by the translocation of the FA transporter FAT/CD36 from intracellular stores to the plasma membrane [1,5]. Contraction-induced FA oxidation is also mediated by a transporter system at the mitochondrial membrane, which includes transport of long-chain fatty-acyl-coenzyme A (CoA) into the mitochondria via carnitine palmitoyltransferase 1. However, the intracellular signaling mechanisms regulating CD36 translocation to the plasma membrane and increased transport of fatty-acyl-CoA across the mitochondrial membrane during contraction in skeletal muscle are not completely defined.

Evidence shows that the signaling molecule extracellular signal-regulated kinase (ERK1/2) may be involved in the regulation of various aspects of contraction-induced FA metabolism [4,6]. Indeed, our laboratory has shown that inhibition of contraction-induced ERK1/2 phosphorylation with the mitogen-activated protein (MAP)/ERK kinase 1/2 (MEK1/2) inhibitor PD98059 prevents the contraction-induced increase in plasma membrane CD36 content and FA uptake during moderate-intensity muscle contraction [4]. These results suggest that ERK1/2 signaling is involved in

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the regulation of FA uptake during moderate-intensity muscle contraction via an increase in CD36 translocation to the plasma membrane. However, to determine the relative importance of ERK1/2 signaling in the regulation of contraction-induced FA uptake during moderate-intensity muscle contraction, a significant linear relationship must be established between ERK1/2 activation and FA uptake as well as with CD36 plasma membrane content. Furthermore, in the above experiments, the effects of ERK1/2 inhibition and the subsequent decrease in FA uptake on contraction-induced FA oxidation were not measured.

Data from recent experiments demonstrate that FA uptake and oxidation as well as ERK1/2 phosphorylation increase from rest to moderate-intensity muscle contraction [7]. Percent FA oxidation was not changed by contraction intensity, suggesting that total FA oxidation was increased in response to the increase in FA uptake that may have been regulated by ERK1/2 activation [7]. In this scenario of cellular signaling, it might be expected that a decrease in ERK1/2 activation during moderate-intensity muscle contraction would be accompanied by similar relative decreases in FA uptake and total FA oxidation. However, we have shown repeatedly that during different experimental conditions, changes in total FA oxidation do not coincide with changes in FA uptake [3,8,9]. This indicates that factors other than FA uptake mediate FA oxidation and that these factors may mediate these changes in FA oxidation independently of FA uptake. In line with this argument, manipulation of FA uptake via different levels of ERK1/2 activation when contraction intensity and other intensity-dependent factors are held constant allows for the evaluation of the role of FA uptake in the regulation of FA oxidation.

Thus, the purpose of this study was to determine the effect of dose-dependent pharmacologic inhibition of ERK1/2 phosphorylation on contraction-induced FA uptake and oxidation to determine (1) whether the relationship between ERK1/2 phosphorylation and FA uptake is linear across different levels of ERK1/2 activation and (2) whether the sequential decrease in ERK1/2 phosphorylation level would be associated with a sequential decrease in total FA oxidation during moderate-intensity muscle contraction. Dose-dependent inhibition of contraction-induced ERK1/2 phosphorylation has been previously achieved with increasing concentrations of the MEK1/2-specific inhibitor PD98059 in a study performed in rat skeletal muscle [10], which made this inhibitor an obvious choice to achieve our purpose. We hypothesized that during moderate-intensity muscle contraction, dose-dependent inhibition of ERK1/2 would lead to a dose-dependent decrease in FA uptake demonstrating the existence of a linear relationship between the 2 variables. We further hypothesized that during moderate-intensity muscle contraction, dose-dependent inhibition of ERK1/2 would not be associated with a dose-dependent decrease in FA oxidation, providing evidence for the notion that factors other than FA uptake regulate FA oxidation.

2. Materials and methods

2.1. Animal preparation

Male Wistar rats (~285-330 g; N = 38) were housed in pairs and kept on a 12:12-hour light-dark cycle. They received standard rat chow and water ad libitum. Rats were randomly assigned to 1 of 4 groups whose hind limbs were perfused with dimethyl sulfoxide and increasing concentrations of PD98059, an inhibitor of the ERK1/2 upstream kinase MEK1/2 [11,12], during moderate-intensity electrical stimulation: 0.0 μmol/L (P1; n = 8), 10 μmol/L (P2; n = 7), 20 μmol/L (P3; n = 7), 50 μmol/L (P4; n = 8). An additional group of animals was perfused at rest in the absence of the inhibitor to serve as control (R; n = 8). All experiments were performed in the early morning during the postabsorptive phase. Ethical approval for the present study was obtained from the Institutional Animal Care and Use Committee at the University of Southern California, Los Angeles.

2.2. Hind limb perfusion

On the day of the experiment, rats were anesthetized and prepared surgically for hind limb perfusion as previously described [7,13]. Before the perfusion, catheters were inserted, and heparin (150 IU) was administered into the inferior vena cava. The rats were killed with an intracardial injection of pentobarbital sodium (0.4 mg/g body weight), and arterial and venous catheters were inserted immediately into the descending aorta and ascending vena cava. The preparation was placed in a perfusion apparatus where the left iliac vessels were tied off and a clamp was fixed tightly around the proximal part of the leg to prevent bleeding [13].

The initial perfusate (250 mL) consisted of Krebs-Henseleit solution, 5% bovine serum albumin (Bovuminar, pH 7; Serologicals, Norcross, GA), 550 μmol/L albumin-bound palmitate, 8 μCi of albumin-bound [1-^{14}C] palmitic acid (MP Biomedicals, Irvine, CA), 8 mmol/L glucose, and vehicle only (dimethyl sulfoxide) or 10, 20, or 50 μmol/L PD98059 dissolved in dimethyl sulfoxide. The inhibitor was added directly to the perfusate with the assumption that this would be the effective inhibitor concentration despite the fact that measurements of inhibitor uptake into the hind limb were not made. Because insulin has been shown to activate the ERK1/2 pathway [10,14], insulin was not included to isolate contraction effects on ERK1/2 phosphorylation and muscle metabolism. The perfusate (37°C) was continuously gassed with a mixture of 95% O_2 and 5% CO_2, which yielded arterial pH values of 7.4 to 7.6 and arterial P_{O_2} and P_{CO_2} values that were typically 320 to 450 and 35 to 52 mm Hg, respectively. Mean perfusion pressures were not affected by PD98059 and averaged 111.5 ± 18.3, 120.3 ± 13.3, 122.6 ± 16.2, 131.0 ± 17.1, and 119.3 ± 22.8 mm Hg in the R, P1, P2, P3, and P4 groups, respectively ($P > .05$).

Hind limbs were equilibrated for 20 minutes and then perfused for an additional 20 minutes at a perfusate flow of 15 mL/min (average for all groups, 0.85 ± 0.01 mL · min^{-1} ·